Studies on the control of russeting in apple fruit with plant growth regulators

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STUDIES ON THE CONTROL OF RUSSETING IN APPLE FRUIT WITH PLANT GROWTH REGULATORS

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ABSTRACT

The factors influencing the initiation and development of russeting and cracking in apple fruit and possible control measures are reviewed.

The potential of gibberellins $A_4 + A_7$ to reduce russeting and cracking of fruit of the cultivars Cox's Orange Pippin, Golden Delicious and Discovery was studied. Applications of ≤ 5 mgl^{-1} GA_{4+7} were found to give marked reductions in russeting and cracking, with little effect of increasing concentration. Multiple applications, with at least four sprays, gave the best skin finish and treatments were most effective when the first spray was applied during the flowering period.

The addition of IAA, NAA or BA to GA_{4+7} did not improve the control of russeting and cracking.

GA_{4+7} increased the size of apple fruit skin cells and altered their periclinal/anticlinal dimensions, while paclobutrazol, a known specific inhibitor of gibberellin biosynthesis, had the opposite effect and increased the incidence of russeting.

An extensometer capable of measuring elasticity and plasticity of very small samples was constructed and used to measure the extensibility of young apple fruit skin tissue. The fruit skin extensibility was found to be increased by GA_{4+7} and decreased by paclobutrazol. This is discussed in relation to the observed effects of the treatments on russeting and cracking.

Uptake and translocation of gibberellins in apple fruit and vegetative tissues was studied using [^{14}C]-GA_3, [^{3}H]-GA_4 and localised applications of GA_{4+7}. Uptake in fruit tissues was rapid and not affected by concentration. Diffusive movement occurred within fruit tissues and there was limited translocation from leaves to fruit.

The implications of the results for the commercial application of GA_{4+7} for the control of russeting and cracking are discussed.

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ABBREVIATIONS

BA	6-benzyladenine
EEx	Elastic extensibility
$[^{14}C]$-GA_3	[1,7,12,18-^{14}C] gibberellin A_3
$[^{3}H]$-GA_4	[1,2(n)-^{3}H] gibberellin A_4
GA_3	Gibberellin A_3
GA_4	Gibberellin A_4
GA_7	Gibberellin A_7
GA_{4+7}	Gibberellins A_4 and A_7
IAA	Indolyl-3-acetic acid
NAA	1-naphthylacetic acid
PEx	Plastic extensibility
SED	Standard error of difference

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INTRODUCTION AND LITERATURE REVIEW

The cultivation of apples in the United Kingdom has a long and varied history, with evidence of apple consumption dating back to the Roman occupation, although this could have been as cider rather than fresh fruit. In more recent times apple growing has experienced several periods both of prosperity and depression, with growers having to face increasingly severe competition from imported fruit from Europe at various times. This was the case in the 16th and 17th centuries, as well as throughout most of the last century, when for example, imported French apples could command in London a price of two or three shillings each (Roach, 1980).

The advice to English growers has always been to grow better quality fruit in order to compete with imports. Bliss wrote in 1825 "... apples grown on diseased trees will not keep and consequently for some years past, our London markets have been principally supplied with foreign apples all through the Spring ..." (Roach, 1980). Today, history is repeating itself, especially since the United Kingdom became a member of the EEC in 1971, and there is now great pressure on growers to produce good quality fruit. Quality requirements are more demanding also because more fruit is marketed through the few major supermarkets chains. The price differential between Class 1 and Class 2 that has developed in recent years is demonstrated for Cox's Orange Pippin in English wholesale markets (Table 1), where Class 1 fruit carried a 70% premium on average.

TABLE 1

Mean seasonal wholesale prices for Cox's Orange Pippin, Class 1 and Class 2. Prices in p/kg, calculated from weekly 'most usual price' published in The Grower October - May. (Adapted from Fenemore and Norton, 1985.)

	1980/81	1981/82	1982/83	1983/84	1984/85	1985/86	1986/87
Class 1	37.3	58.3	41.0	54.0	49.5	61.4	48.4
Class 2	26.0	46.5	29.4	38.0	33.3	41.2	30.1
Difference	11.3	11.8	11.6	16.0	16.2	20.2	18.3
Class 2 price as a percentage of Class 1 price	69.7%	79.8%	71.7%	70.4%	67.3%	67.1%	62.2%

The figures demonstrate clearly the financial importance to the grower of producing Class 1 fruit if they are to maintain a profitable business.

The components of fruit quality include fruit size, colour and skin finish of the fruit, all of which are important in the case of Cox's Orange Pippin, the major dessert apple grown in England. Eight thousand hectares account for 60% of the total area of dessert apples (MAFF, 1987). The greatest single cause of downgrading Cox's Orange Pippin and other susceptible cultivars, can be russeting and cracking of the fruit skin (Skene, 1982). Good appearance of fruit has increased in importance as increased emphasis on fruit quality has developed, which in turn has led to greater economic pressure to understand and if possible control russeting of apple fruit.

1.1 STRUCTURE OF THE FRUIT OF APPLE

An apple is a fleshy fruit consisting of five carpels surrounded

by a fleshy receptacle. The innermost region surrounding the carpels, known as the pith, is limited on the outside by the core line, a ring of 10 vascular bundles. Outside the core line is a parenchymatous region, the cortex, which is contained within the skin of the fruit. The skin comprises a hypodermal layer several cells thick, a single layer of cells forming the epidermis, and a wax-like outer covering, the cuticle. The general morphology and histology of the developing fruit has been described by Tukey and Young (1942), with specific studies on the skin tissues by Tetley (1931); Bell (1937a); Meyer (1949); De Vries (1968); Faust and Shear (1972b) and Miller (1982).

1.1.1. Cuticle

The cuticle is approximately 1-2µm in thickness at blossom time, increasing to 10-25µm by harvest time, depending on the cultivar (Tukey and Young, 1942; Meyer, 1944; Miller, 1982). It can be laid down as a relatively uniform layer as in the cultivars McIntosh Red, Bramley's Seedling and Jerseymac (Tetley, 1930; Bell, 1937a; Miller, 1982), or as a highly irregular layer as in Golden Delicious, Jonagold and Cox's Orange Pippin (Tetley, 1930; Meyer, 1944; Miller, 1982). During the course of fruit growth in many cultivars the cuticle extends inward between the epidermal cells to form 'wedges' or 'flanges'. By the end of fruit development, these flanges can reach the hypodermis and completely envelop the epidermal cells. This process contributes to the irregular nature of the cuticle found in the cultivars mentioned earlier.

Miller (1982) studied the development of cuticular flanges in fruit of 20 apple cultivars and in general found them to be perceptible

a few weeks after anthesis, with flange thickness ranging from 2.5-15μm and length from 11-42.5μm at harvest. It is suggested that they have a role in cuticle anchorage (Kolattukudy, 1980).

The structure of the cuticle has been described by Miller (1982) as "... an amorphous, optically isotropic, negatively birefringent, non-lamellated entity", in contrast to that reported for many leaf cuticles. Although Hilkenbaumer (1958) and Linskens and Gelissen (1966) found the cuticle to be periclinally lamellated in fruit of Ontario and Golden Delicious respectively, Miller (1982) found no evidence in his study of ten varieties for such a structure. He did find, however, evidence of the presence of transcuticular canals which permeated the cuticular matrix and terminated as discrete pores on its outer and inner periclinal surfaces.

1.1.2. Epidermis

At anthesis the epidermal cells are arranged as a single layer of radially elongated cells, these becoming more rectangular in shape as fruit growth progresses (Bell, 1937a; Meyer, 1944). Although a distinct epidermal layer is recognisable in fruit of some cultivars at harvest eg McIntosh Red (Bell, 1937a), the occurrence of frequent periclinal divisions in the epidermal cells and resulting invasion of the epidermis by the cuticle in others eg Golden Delicious and Cox's Orange Pippin, results in the loss of identity of the epidermal layer in these cultivars by harvest (Meyer, 1944; Skene, 1962).

Cell division in the epidermis continues for a longer period than in the cortex; according to Bell (1937a) and Tetley (1931) cell division had ceased by the end of June in the epidermis of McIntosh and

Bramley's Seedling respectively, whereas Tukey and Young (1942) considered that in McIntosh Red and Twenty Ounce cell division continued until the end of August. Skene (1966) in his study of Cox's Orange Pippin, determined that cell division stopped when fruits were about 45mm in diameter or about 65-70 days after blossom ie late July - early August. This apparent conflict may be due to cultivar or climatic differences, but also to the different techniques used by the authors to determine when cell division had ceased.

1.1.3. Hypodermis

The hypodermal cells at flowering are slightly smaller than those of the cortex but cannot be distinguished from them by shape or cell wall thickness (Tukey and Young, 1942). Soon after flowering the cell walls begin to thicken and they come to form a dense mass of irregular closely-packed, radially-flattened cells (Bell, 1937a; Tukey and Young, 1942).

Skene (1962) reported that the hypodermal cells of Cox's Orange Pippin divide periclinally more often than the epidermal cells during the early stages of fruit growth. Anticlinal divisions occurred more frequently at a later stage when periclinal division had become less apparent, although due to the large changes in shape of the hypodermal cells during the development of the fruit, it was impossible to be conclusive as to the time at which all division ceased.

1.2. RUSSET AND CRACKING OF APPLE FRUIT

Definitions

Russet can be defined as a periderm that replaces epidermal tissue and forms a continuous layer of protective tissue on an apple fruit

surface (Skene, 1982).

Russet is sometimes covered with shallow cracks, up to 1mm deep and is referred to as rough russet; deeper cracks or cracks not associated with russet are referred to simply as cracks or cracking.

1.2.1. Initiation and Development

Zschokke (1897) observed that russet originated in the epidermis of the fruit and Bell (1937b) in his study of the Golden Russet apple confirmed this observation. He noted that the development of the skin tissues was very similar to that found in McIntosh Red (Bell, 1937a), up to and during flowering, after which variations in the developmental processes appeared. The key difference was found to be the occurrence of periclinal divisions in the epidermal cells, which started during flowering and continued until nearly the whole epidermis became transformed into a layer two cells thick by the middle of June. During the first two weeks of July the epidermal layer differentiated very rapidly to produce an active cork cambium (phellogen) in the innermost epidermal cells. During the development of the periderm the outer cells of the epidermal layer died, followed by rupture of the cell walls and the cuticle, to give the first visible indication of the rough but finely textured surface typical of a russet apple.

More recent studies by Simons (1960, 1962, 1965), indicated a different development of russet in russet sports of Golden Delicious. Periclinal divisions of the epidermal cells were observed shortly after flowering, and whereas cell division continued in the fruit of the normal Golden Delicious, it had ceased 14 days after flowering in fruit of the russet sports. This lack of cell division in the epidermal

layer resulted in the inability of the fruit of the russet sports to increase in size without a disruption of the outermost protective layers. When this occurred, a wound response led to the formation of a phellogen and subsequent development of russeting.

As mentioned earlier the phellogen can originate in the epidermis but there is also evidence of the involvement of the hypodermis in some cases. Thus Pratt (1972) observed that the phellogen arose in the hypodermal layers of the fruit of the russet sport Stark 287, this following extensive periclinal divisions of the hypodermal cells and subsequent death of the cells of the epidermis and outer hypodermis. Russet initiation was also found to involve the hypodermis in fruit of Cox's Orange Pippin, where a phellogen was evident by early June (Sironval and Clijsters, 1962). Apparently russet can be initiated either in the epidermis, hypodermis or both depending on the cultivar, although the actual stimulus causing russet formation may also have a bearing on which cells are involved.

In a survey of 66 apple cultivars Skene (1962) found a correlation between the amount of russet on the surface of the fruit at harvest and the presence of dead cells in the epidermis and fine cracks in the cuticle of the fruit in the first few weeks after flowering. Histiological examination showed that cell division could take place below a dead cell or cuticle crack, such cell divisions being typical of those that are associated with russet initiation. However, since some cultivars that developed russeting had virtually no dead cells or cuticle cracks in the fruit, Skene concluded that either dead cells or cuticle cracks are not directly responsible for russet initiation, or some additional factor must be involved.

Other workers (Watanabe, 1969; Ashizawa et al, 1984) have concluded that cracks in the cuticle, forming in the period immediately after flowering, are the cause of russet initiation in susceptible cultivars such as Golden Delicious. There is conclusive evidence that the skin of Golden Delicious fruit tends to develop cracks in the cuticle at an early stage of fruit development (Meyer, 1944), and that these are more prevalant than in the fruit of apple cultivars that are not prone to russeting (Faust and Shear, 1972b).

The relationship between cracks in the cuticle and russet initiation was mentioned by Brown and Koch (1962), in relation to the occurrence of stalk-end russeting in Yellow Newtown apple. They found that tangential elongation of the epidermal and hypodermal layers in the stalk cavity led to the cells having a 'stretched' appearance and that this, coupled with fewer radial divisions of the epidermal cells, resulted in cuticle cracking in this region as the fruit developed. 'Stretched' hypodermal cells were also observed by Skene (1962) in relation to the incidence of russeting at the stalk-end of fruit of Cox's Orange Pippin, which was higher in this region compared to other areas of the fruit surface.

It would seem from the published studies that russeting can arise in two ways depending on the apple cultivar involved. In totally russeted cultivars eg Golden Russet, a periderm is formed in the epidermal and/or hypodermal layers due to their disruption during fruit growth, usually preceded by periclinal divisions in the epidermal cells. In apple cultivars which are susceptible to russeting but are potentially smooth-skinned eg Cox's Orange Pippin and Golden Delicious, lack of growth in the epidermal and hypodermal regions of the fruit

leads to stresses developing during growth which results in cuticle cracking and subsequent russet formation. However, it is still not clear whether cracking of the cuticle represents the first or last stage of russet development (Skene, 1982). The cracks could stimulate the formation of a periderm as a wound response or, alternatively, a periderm may form under an intact cuticle, reducing the capacity of the cuticle and epidermis to expand with the fruit and resulting in subsequent cracking of the cuticle as growth continues.

1.3. FACTORS INFLUENCING THE INITIATION AND DEVELOPMENT OF RUSSETING

1.3.1. Genetics

There is considerable variation in the extent to which different apple cultivars develop russet, from smooth skin to total surface cover. In the case of totally russeted cultivars eg Egremont Russet, there is evidence for simple genetic control; crossing between heavily russeted cultivars gave distinct segregation for russeting in the progeny (Alston, 1973). Other varieties such as Cox's Orange Pippin and Golden Delicious are potentially smooth skinned apples but which have the tendency to develop russeting under certain conditions, these cultivars being defined as partially russeted cultivars. Evidence from breeding work involving Cox's Orange Pippin and its derivatives support the hypothesis that a major dominant gene, the effects of which are modified by minor genes, is involved with these varieties. In other words, partial russeting is polygenically controlled (Alston and Watkins, 1975).

There is considerable evidence for variation in the susceptibility to russeting within partially-russeted varieties. In both Cox's Orange

Pippin (Campbell, 1973) and Golden Delicious (Cummins et al, 1977; Sansavini and Bassi, 1977; Oosten et al, 1982), clones exist that are more or less susceptible to russeting than the standard cultivar itself. 'Smoothee' is one such clone of Golden Delicious which has consistently produced less russeted fruit than the standard Golden Delicious, whereas various spur-type clones produce consistently fruit with more russet (Cummins et al, 1977). Commercially, there is considerable advantage in growing clones with inherently smoother skinned fruit and much effort has gone into looking for such clones. Additionally, treating dormant vegetative material with gamma radiation is a technique used currently to produce mutations which may posess improved characteristics (Decourtye, 1967; Lappins, 1971; Lacey, 1982).

1.3.2. Fruit Morphology

Russeting of some varieties is only partial and there are differences in the amount of russet associated with various areas of the fruit. Russeting of Yellow Newtown apple is confined to the stalk cavity (Brown and Koch, 1962) and Skene (1962, 1982) also found the occurrence and severity of russeting to be most prevalent in the stalk cavity of Cox's Orange Pippin. In both cases it was found that differences existed in the epidermal and hypodermal layers between the stalk cavity and the main body of the fruit with cells being tangentially (periclinally) elongated in the former. This was taken as evidence of greater stress in this portion of the fruit during growth and to be correlated with the greater amount of russet in the stalk cavity.

1.3.3. Position of the Fruit Within the Cluster

As a general rule it has been found that in partially-russeted

varieties, the 'King', the fruit arising from the terminal flower of the inflorescence has less propensity to russet than the laterally-borne fruits (Brown and Koch, 1962; Skene, 1962; Lotter and Van Zyl, 1964; Watanabe, 1969). In his study of russeting in Cox's Orange Pippin, Skene (1962) found that the 'King' fruits were the least russeted on all areas of the fruit surface, but that only the differences between the stalk end of the fruits were statistically significant. Variation in the amount of cracking between 'King' and lateral fruits was restricted to the stalk end of the fruits, the former being less cracked. Skene found that these differences could not be explained in terms of differences in the distribution and rate of growth over the fruit surface. For example, he found the growth rate of the stalk end of 'King' fruits to be the same as that of lateral fruits and concluded that the differences were probably due to differences in fruit shape, since 'King' fruits are generally more elongated and have a shallower stalk cavity with a shorter pedicel than lateral fruits (Westwood and Blaney, 1963; Watanabe, 1969).

Brown and Koch (1962) associated differences in russeting between 'King' and lateral fruits of Yellow Newtown apple with differences in the epidermal and hypodermal regions of the fruit, although Watanabe (1969) found no such differences in the five apple cultivars he studied and which also exhibited a similar distribution of russeting.

1.3.4. Climate

There is considerable evidence that environmental factors can play a major part in determining the extent and severity of russeting that develops in a partially russeted variety in any particular orchard and year (Walter, 1967; Faust and Shear, 1972a). Among the various

environmental factors it seems that rainfall and/or humidity play a crucial role in determining the degree of russeting and the variation from year to year.

1.3.5. Rainfall

There are conflicting reports on the influence of rainfall. Chandler and Mason (1942) found no significant evidence for a relationship between rainfall and russet formation in Golden Delicious, while Dalbro (1958) found that the incidence of russeting in Cox's Orange Pippin in different years was closely related to the number of rainy days, and Montgomery (1959) associated the widespread russeting and cracking of the same variety in England in 1958 with exceptionally heavy rainfall during June and August.

Additional evidence for a relationship between rainfall and russeting comes from a number of studies in which fruits have been protected from rain by means of shelters erected over trees or by bags placed over the fruits. In most cases shelters were constructed using polyethylene film and covered the fruit for various periods starting at blossom time or soon after. The incidence of russeting was found to be reduced in all cases with both Golden Delicious and Cox's Orange Pippin compared to unsheltered controls (Dalbro, 1958; Hatch, 1975; Creasy and Swartz, 1981; Forsline et al, 1983). Thus Creasy and Swartz (1981) found that russeting was reduced in Golden Delicious if fruits were covered from twenty days after full-bloom until harvest, but covering from one day after full-bloom had significantly greater effect, which points to the immediate post-bloom period as a critical time when russet can be initiated and when control measures should be applied.

Covering fruit with paper bags from the immediate post-bloom period until harvest has also been shown to reduce russeting (Tukey, 1959; Kanbe et al, 1973; Ashizawa et al, 1984), and is a method used in Japan on a commercial scale to produce russet free fruit (Klackle, 1978).

1.3.6. Humidity

Experimental evidence for the effects of high relative humidity on the incidence of russeting has been provided by several workers who used plastic or impermeable bags to cover fruit (Tukey, 1959; Watanabe, 1969). In all cases russeting was increased where fruit was enclosed in polyethylene or bags made from other impermeable material. Tukey (1959) enclosed fruits of Golden Delicious and Rome Beauty in bags made of various materials from approximately three weeks after petal-fall until harvest. Both varieties developed severe russeting when enclosed in polyethylene bags irrespective of the position of the fruit on the tree. In contrast fruit enclosed in paper or bags made from Ethocel, (permeable to water and light), had excellent skin finish with very little russet, although there was evidence of many small cracks in the cuticle. Other environmental factors would also be influenced by the bags such as temperature and light but Tukey considered that the most important factor influencing russet initiation was relative humidity. It is interesting to note that Skene (1962) covered whole trees of Laxton's Fortune with a polyethylene tent, similar to the rain shelters referred to previously, except that the trees were totally enclosed. The temperature and relative humidity levels were high within the tent and this fruit developed severe russet compared to fruit on uncovered trees. Skene found that a periderm had formed before rupture of the

cuticle and was associated with groups of dead epidermal cells, although it was not clear whether these had initiated periderm formation or vice versa.

In his study of the relationship between various weather parameters with russeting of Golden Delicious over an eight year period, Creasy (1980) found the highest correlation to be with relative humidity. The correlation was highest for the five day period between sixteen and twenty days after full-bloom, this giving a further indication of the critical nature of the period immediately after flowering.

1.3.7. Light/Shade

The effect both of the quantity and spectral composition of light on the incidence of russeting is far from clear, primarily because of the difficulty in separating the independant effects of light and rainfall. Any experiment designed to alter the quantity or quality of light incident on orchard trees will almost certainly have some effect on the exposure of the fruit to precipitation and will thus confound the interpretation of results.

The first worker to report a relationship between light and russeting is Zschokke (1897), who observed that if apples of a cultivar normally prone to russeting are shaded early in the growing season, they remain free of russet. Watanabe (1969) had made the general observation that the incidence of russeting is greater in fruits of Jonathan and Golden Delicious from the exposed parts of the tree, compared to those from shaded areas.

Jackson et al (1977) showed that shading Cox's Orange Pippin trees

from post-bloom to harvest reduced the incidence and severity of russeting and cracking that developed. Trees were shaded to receive varying proportions of full daylight and it was found in all three years of the experiment, that the trees under all levels of shading produced fruit with a better skin finish compared with fruit from unshaded controls. Fruit from trees under the most severe shading treatment were the least russeted.

Long (1980) shaded Golden Delicious trees from petal-fall until forty days later and found fruit was significantly less russeted from the shaded trees. He admitted, however, that the shading material used could have led to an increased temperature and/or have given some protection from rainfall and that this would have confounded the shading effects.

Further indirect evidence for the effects of shade on russeting comes from rootstock/spacing trials. Russeting and cracking of Cox's Orange Pippin has been found to be inversely related to the vigour of the rootstock in trials in Belgium (Monin, 1961), Denmark (Dalbro, 1958) and England (Skene, 1982). Trees on more vigorous rootstocks tend to be larger with a denser canopy and hence produce a greater degree of shading of the fruit compared to that produced on more dwarfing rootstocks.

Russeting of Cox's Orange Pippin has also been found to be inversely related to between row spacing of the trees, suggesting that the more intense shading of closer trees gives rise to less russeting (J W Palmer, unpublished).

Histological examination of the skin tissues from the shaded and

exposed portions of apple fruit have shown that cuticle thickness is generally greater on the shaded portion (Baker, 1930; Shutak and Schrader, 1948; Knuth and Stosser, 1987). Verner (1938), however, suggested that Stayman Winesap fruit growing in dense shade of the innermost parts of trees had thinner skins than those from more exposed parts of the tree; it may be relevant that general observations were made on the skin tissues as a whole rather than the cuticle in particular.

Knuth and Stosser (1987) found that the structure of the surface of the cuticle varied between the exposed and shaded portions of the fruit, when viewed under a scanning electron microscope. The surface waxes had a crystalline platelet structure on the exposed side, compared to the shaded side where waxes formed amorphous 'droplet' structures.

Attempts have been made to evaluate the effects of light quality on russeting, using coloured cellophane materials to construct bags or open-ended cylinders which covered the fruit. Watanabe (1969) covered fruits of Golden Delicious, Jonathan and Ralls with bags made of blue, red and clear cellophane but did not appear to reach any definite conclusions. Long (1980) used open-ended cylinders constructed of red, blue, purple, green, yellow, orange and clear cellophane to cover Golden Delicious fruit from petal-fall until forty days later. The results showed that russeting was significantly reduced by the red, blue, purple and clear cellophane treatments compared to uncovered controls, although all cellophane treatments produced fruit with better skin finish than the controls. The blue cellophane treatment produced the least russeted fruit. Treatments also affected the components of

the cuticle but no significant correlations were found between these effects and the incidence of russeting. Long concluded that the effects on russeting of the various treatments were probably not due to variations in light quality but more probably could be accounted for by the partial sheltering effects of the cylinders in reducing exposure to rainfall.

1.3.8. Mineral Nutrition

Both Eggert and Mitchell (1966) and Stubbings and Strydom (1965) found a positive relationship between nitrogen application and russeting of Golden Delicious fruit. Other observations include a high incidence of russeting and cracking of Cox's Orange Pippin with high potassium applications (Greenham, 1965) and conversely, increased russeting of several varieties associated with potassium deficiency (Dalbro, 1958). Eggert and Mitchell (1966) also found that high potassium applications produced fruit with a better skin finish, whereas high magnesium levels were associated with increased russeting.

In a study designed to examine the effects of mineral nutrition on russeting of Golden Delicious, Hatch (1975) used trees grown in large containers and treated them with nutrient solutions of varying mineral composition. Results showed positive and negative correlations between magnesium and potassium and fruit russet, mineral levels being measured in leaves sampled in July and August. No correlations were found, however, between russeting and the mineral composition of the fruit at harvest. No significant differences were found in the mineral composition between russeted and non-russeted fruits and Hatch considered that this result, coupled with the results from the nutritional studies, proved that mineral nutrition has little effect on

the incidence of russeting under most conditions.

1.3.9. Water Supply

Results from surveys in Europe have shown that russeting of Golden Delicious tended to be more prevelant in areas where trees suffered from fluctuating water supplies during the growing season (refs. cited in Walter, 1967). Catzeflis (1979) showed that russeting of Golden Delicious is increased where trees suffer water stress in the spring. Irrigation of trees has also been shown to reduce the incidence of russeting in Golden Delicious in some trials (Noteboom, 1976; Goode et al, 1978). Katschner (1978) observes that it would seem advisable for the grower to ensure that trees neither become waterlogged nor water-deficient during the growing season in order to reduce the risk of russeting.

1.3.10. Damage Induced Russeting

A periderm can be initiated when the skin tissues of apple fruit are physically damaged, as demonstrated by experiments in which fruits have been mechanically wounded (Skene, 1981). Russeting can be induced by many factors which cause such injury.

It is well documented (Simons, 1957, 1969; Simons and Lott, 1963; Simons and Doll, 1976) that frost during the flowering period can cause extensive damage to or death of epidermal/ hypodermal cells, which results in the formation of a periderm and development of russet.

In recent years it has been recognised that russeting can be caused by the apple rust mite Aculus schlechtendali, this first being reported by Ciampalini et al (1976) in Italy, with subsequent reports

of damage in England (Robinson and Winfield, 1981) and Holland (van Epenhuijsen, 1981). In an extensive study of the relationship between apple rust mite and russeting in four apple cultivars, Easterbrook and Fuller (1986) found significant correlations between the numbers of rust mites feeding on fruitlets shortly after flowering and the amount of russet on the fruit at harvest. Histological studies showed that the feeding of the mites on the flower receptacles and/or fruitlets in May and June caused epidermal cell injury, resulting in the formation of a phellogen and subsequent russeting of the fruit.

Russet can be due also to infection of the fruit by various fungal diseases if these are present at the early fruitlet stages. This has been shown following infections by Alternaria mali (Mizuno and Takahashi, 1978) and apple powdery mildew Podosphaera leucotricha (Zorbrist, 1962). Zorbrist showed that it was the perforation of the epidermis by the hyphae from germinating spores that caused a wound response and russet development.

Any substance that is sufficiently toxic to damage epidermal cells when sprayed onto a fruit, may result in russet formation. A number of reports in the literature refer to such spray induced russet covering a wide range of organic and inorganic substances, including insecticides, fungicides and iron-contaminated water . The reviews by Faust and Shear (1972a) and Walter (1967) give a total of 36 references on the subject and the specific area of fungicides and russeting has been reviewed by Agnihothrudu et al (1983).

Among those reports not covered by the three reviews above include those mentioning increased russeting after sprays of carbaryl (Link, 1973; Comai and Widmann, 1978), diazinon (Creasy and Swartz, 1981),

oxamyl (Byers, 1978; Meyer, 1982), antitranspirants (Byers et al, 1983) and surfactants (Noga and Bukovac, 1986; Richardson et al, 1986).

The effects of many of these substances on russeting is often variable due to the large number of other factors that can influence the susceptibility of the fruit to damage. These include the weather conditions during and immediately after the spray application (Palmiter, 1944; Kirby and Bennett, 1967), and more importantly, the stage of fruit development when sprays are applied, with the immediate post-blossom period appearing to be the most sensitive time (Bondoux et al, 1971).

1.4. FACTORS INFLUENCING THE INITIATION AND DEVELOPMENT OF CRACKING

As referred to earlier cracks in the cuticle are considered the main cause of russet initiation in apple fruits; cracking in this form is inseparably linked with russeting. Cracking of certain apple cultivars can occur, however, on smooth skin and be completely unrelated to russeting. Varieties in which this type of cracking is known to occur include Cox's Orange Pippin (Skene, 1962, 1982), Discovery (Joosse, 1982), Stayman Winesap (Verner, 1935, 1938; Gardner and Christ, 1953) and York Imperial (Shutak and Schrader, 1948).

1.4.1. Rainfall

The effect of rainfall on cracking of apple fruit has been related generally to fluctuations in rainfall during the growing season which cause rapid changes in the water relations of the soil and the tree. Heavy rainfall late in the growing season was cited by Fisher (1937) as being the major cause of an increased incidence of cracking of York Imperial apples. Goode et al (1975) described cracking of the skin of Cox's Orange Pippin fruit and related this to a dry August period

followed by heavy rainfall in September, which caused a surge in fruit growth.

Verner (1935) attempted to simulate the conditions caused by fluctuating rainfall by artificially droughting Stayman Winesap apple trees, followed by flood irrigation. The incidence of cracking was not increased by this treatment and he concluded that neither changes in soil-moisture content caused by rainfall, nor any direct effect of the rainfall itself was responsible for cracking in this variety.

1.4.2. Humidity

Verner (1935) found a positive association between low rates of evaporation and the incidence of cracking in Stayman Winesap fruit, this being extensive during periods of prolonged slow evaporation rates. He concluded that cracking was initiated by increased water supply to the fruit as a result of reduced transpiration under conditions of high humidity.

1.4.3. Light/Shade

The effects of shade on the incidence of russeting and cracking in Cox's Orange Pippin have been referred to eariler. Verner (1938) noted a similar relationship between shade and cracking of Stayman Winesap apples. Fruit from the inner, most densely shaded parts of the trees had very few cracks compared to those from the more exposed parts. He also found that brown paper bags reduced the incidence of cracking if placed over fruit three to four weeks before harvest.

In contrast, Shutak and Schrader (1948) found that the incidence of cracking of York Imperial apples was higher on the green, shaded areas of the fruit surface compared to the exposed areas. The cuticle

morphology was also found to vary in relation to fruit exposure, the shaded side having a thick, irregular cuticle compared to the thinner, regular cuticle of the exposed side. It should be noted, however, that cracking of York Imperial fruit tends to be superficial and confined to the uppermost layers of the skin tissues, in contrast to the deeper cracks that can occur in other varieties. Knuth and Stosser (1987), found that fine cracks in the skin of four apple cultivars were more evident on the shaded side of the fruit and that the cuticles were thicker in these regions.

1.4.4. Mineral Nutrition

The incidence of cracking in apples has been shown to increase when boron is deficient in the fruit tissues. This was demonstrated with Egremont Russet by Dixon et al (1973), who showed that three boron sprays applied at petal fall and fourteen and twenty-eight days after petal-fall, completely prevented cracking of the fruit in the two years of the experiment. Soil analysis showed that although boron levels appeared adequate for normal tree growth, levels were deficient in the fruit tissues of the untreated controls. Soil applications of boron had little effect on the incidence of cracking, suggesting inadequate uptake.

It appears that sprays of boron are only effective in reducing the incidence of cracking where levels in the fruit tissues are deficient. This was shown by Yogaratnam and Johnson (1982), who found that sprays of boron either had no effect or increased the incidence of cracking in Cox's Orange Pippin and Discovery. The levels of boron in the leaves and fruit of untreated controls was found to be normal and to have been increased by the boron applications. It is possible, therefore that

either low or high levels of boron in the fruit tissues can increase the incidence of cracking in the fruit.

1.5. TECHNIQUES TO REDUCE THE INCIDENCE OF RUSSETING AND CRACKING OF APPLES

1.5.1. Cultural

There are many steps that growers can take in orchard management to reduce the risk of russet and cracking developing in the fruit (Vogl, 1985). General considerations are to plant in good, well sheltered sites with good soil, taking care to correct any mineral deficiences and soil drainage problems. Where available, the grower should plant clones of the variety which are known to be less susceptible to russeting and cracking. For example, in Cox's Orange Pippin, 'Queen Cox' has been shown to produce fruit with a better skin finish (Clarke, 1984). Effective pest and disease control programmes should be used, avoiding the use of any chemical during the critical post-bloom period that may cause russeting and where possible using irrigation to avoid water stress and using frost protection measures during the flowering period.

1.5.2. Spray Applications

Although enclosing fruit in paper bags controls russeting in Golden Delicious and is a technique used commercially in Japan to ensure good quality fruit for market (Klackle, 1978), such a practice is not considered economic elsewhere. Consequently effort has concentrated on the possibility of economic spray treatments and numerous chemicals have been evaluated for this purpose.

1.5.2.1.a. Fungicides

Sprays of both captan and the product Tuzet (mixture of thiram, ziram and dithiocarbamate) have reduced the incidence of russeting in Golden Delicious (see refs. cited in Walter, 1967; Faust and Shear, 1972).

1.5.2.1.b. Insecticides

The incidence of russeting in Golden Delicious was decreased when sprays of the insecticide dimethoate were applied in the immediate post-blossom period (Kremer, 1967; Skene, 1980b; Steenkamp et al, 1984). Its effectiveness varied, however, with some trials yielding no treatment effects. It is possible that the beneficial effects that occurred were indirectly due to control of apple rust mite Aculus schlechtendali, which is known to be a causal agent of russeting (Easterbrook and Fuller, 1986). However, no evidence of the mode of action of dimethoate in russet control has yet been published.

1.5.2.1.c. Miscellaneous Compounds

There is considerable evidence that the application of the product Golclair can reduce the incidence of russeting in Golden Delicious if applied as several sprays in the immediate post-bloom period (Cobianchi and Francesconi, 1973; Rui, 1975; Cesari and Francia, 1979; Steenkamp et al, 1984). Golclair consists of 60% sulphur, 1.8% boron and 15% silica aluminate. Another product containing boron, Maneltra-Borium, was evaluated for russet control in Golden Delicious and Karmijn de Sonnaville but the results obtained were variable (Wertheim, 1980).

The application of compounds containing silicon dioxide in the immediate post-bloom period has been shown to significantly reduce the incidence of russeting in Golden Delicious (Edgerton et al, 1976;

Meador, 1977; Edgerton and Veinbrants, 1979; Wertheim, 1980; Creasy and Swartz, 1981; Steenkamp et al, 1984). Trials showed that a single spray containing 2.5% of the product Apasil applied at petal-fall reduced russeting significantly and that a second spray applied eight to ten days later gave a further improvement in skin finish.

The mode of action of compounds containing silicon dioxide has been attributed to the formation of a layer over the fruit surface which protects the fruit from the effects of rainfall and/or humidity (Edgerton et al, 1976). Trials with other compounds, selected for their film coating forming properties in an attempt to restrict contact between the fruit surface and external water, have produced variable results (Byers et al, 1983). Some compounds were found to reduce the incidence of russeting in Golden Delicious but others gave rise to the reverse effect.

1.5.2.2. Plant Growth Regulators

1.5.2.2.1. Auxins

There is evidence that synthetic auxins can reduce the incidence of russeting in Golden Delicious if applied soon after flowering. Most of this evidence comes from trials using the synthetic auxin naphthylacetamid (NAAm) as a fruit thinning agent (Schumacher and Fankhauser, 1967;Link, 1973; Schumacher et al, 1977).

Further evidence for the potential of synthetic auxins to control of russeting has arisen from the use of 2-(2,4,5 - trichlorophenoxy)-propionic acid (2,4,5-TP). Single or multiple applications, at concentrations ranging from 1 to 20 mgl^{-1} a.i. were applied soon after flowering, all treatments reducing russeting significantly compared to the controls (Byers et al, 1983). Other treatment effects included a

reduction in both fruit size and number of seeds per fruit, especially when the higher concentrations were used.

1.5.2.2.2. Gibberellins

Wertheim (1971) first reported that gibberellins could reduce russeting and cracking in apples; his work showed that a mixture of the gibberellins A4 and A7 (GA_{4+7}) had reduced drought - induced cracking in Cox's Orange Pippin fruits. Reduction in the incidence of russeting after GA_{4+7} applications was first reported by Eccher (1975) in Golden Delicious and by Taylor (1975) in Golden Delicious and Jonathan . Subsequent reports have been published by workers in Australia (Taylor, 1978); Canada (Elfving and Allen, 1987); Italy (Cobianchi and Bagnara, 1983; Eccher, 1978, 1983; Eccher and Boffelli, 1978, 1981); Holland (van Dijke and Kester, 1983; Joosse, 1982; van Rooijen, 1983; Scholtens and Bootsma, 1981; Wertheim, 1980, 1982; Westerlaken, 1982); South Africa (Steenkamp et al, 1984) and the United States of America (Edgerton and Veinbrants, 1979, Meador and Taylor, 1987). These have covered various aspects including concentration, timing of application, number of sprays and spray interval, together with assessments of side effects of GA_{4+7} applications. The work has concentrated on Golden Delicious but other cultivars mentioned include Cox's Orange Pippin, Discovery and Karmijn de Sonnaville. Gibberellic acid has some effect on russeting but to a lesser extent than GA_{4+7} (Eccher, 1978; Eccher and Boffelli, 1978 ; Taylor, 1978; Wertheim, 1982).

An inverse relationship between GA_{4+7} concentration and the incidence of russeting has been demonstrated (Taylor, 1975, 1978; Elfving and Allen, 1987; Meador and Taylor, 1987), although other results have shown an inconsistent response (Eccher and Boffelli, 1978,

1981; Scholtens and Bootsma, 1981; Westerlaken, 1982). Other factors such as the timing of spray applications (Eccher and Boffelli, 1978, 1981) and the cultivar (Westerlaken, 1982) may interact with concentration. Indeed Eccher and Boffelli (1981) concluded that timing and number of sprays of GA_{4+7} were more important than concentration for the control of russeting.

As previously described, the immediate post-bloom period is critical and it is not surprising therefore that trials results show that applications of GA_{4+7} during this period are optimal for the control of russeting. In comparing spray programmes that start at flowering or petal-fall and those starting later than petal-fall, the former gave consistently better results and programmes starting well after petal-fall often had little or no effect on the incidence of russeting (Eccher, 1978, 1983; Eccher and Boffelli, 1978, 1981; Taylor, 1978; Elfving and Allen, 1987). For example, a trial which compared spray programmes applied from six days before full-bloom to twenty-two days after showed that programmes starting eight or fifteen days after full-bloom gave the greatest reduction in russeting, and that there was no effect where the first spray was applied twenty-two days after full-bloom (Eccher and Boffelli, 1981).

Other trials have compared spray programmes starting at the beginning of flowering and programmes starting at petal-fall, but with varying results giving no overall conclusion (Westerlaken, 1982; van Rooijen, 1983;).

There are many reports that multiple spray applications of GA_{4+7} result in greater reductions in the incidence of russeting than single or lower numbers of sprays (Eccher, 1978; Eccher and Boffelli, 1978,

1981; Elfving and Allen, 1987). These results are confounded, however, by the variable amount of GA_{4+7} applied. Nevertheless, in trials where the concentration of GA_{4+7} was varied to compensate for numbers of sprays, Schlotens and Bootsma (1981) demonstrated clearly the benefits of multiple spray applications. The results showed that four sprays of 10 mgl^{-1} GA_{4+7} resulted in significantly less russeting than either two sprays of 20 mgl^{-1} or one spray of 40 mgl^{-1} GA_{4+7}. In other trials where programmes of four, six and eight sprays at ten day intervals were compared, four sprays of 10 mgl^{-1} GA_{4+7} gave better control of russeting than eight sprays of 2.5 mgl^{-1} (Westerlaken, 1982). However, the results suggested that a further factor, the period of time over which spray programmes are applied, also confounded the results, since eight sprays would be applied over a considerably longer period of time than four sprays.

This factor was taken into account in trials reported by van Rooijen (1983) and Dijke and Kester (1983), whereby the concentration of the GA_{4+7} sprays and the spray intervals were varied, in order that the same total amount of GA_{4+7} was applied over the same time period, irrespective of the varying number of sprays. Results showed that a spray programme of six sprays of 6.5 mgl^{-1} GA_{4+7} at seven day intervals controlled russeting more than a programme of four sprays of 10 mgl^{-1} GA_{4+7} at ten day intervals, both on Golden Delicious and Karmijn de Sonnaville.

An additional consideration that influences the concentration of GA_{4+7} which may be used in orchards for russet control, is the risk of detrimental side effects such as suppression of return bloom, increased shoot growth, reduced fruit set and number of seeds per fruit.

Since the early 1960's it has been known that spray applications of high concentrations of gibberellins, including GA_3 and GA_{4+7}, can inhibit the flowering of apple trees in the year after application (Guttridge, 1962; Marcelle and Sironval, 1963; Dennis and Edgerton, 1966; Tromp, 1973). A general inverse relationship between number of flowers produced in the year following treatment and GA_{4+7} concentration has been noted (Taylor, 1978; Meador and Taylor, 1987). In other trials Wertheim (1982) noted a significant reduction in return bloom in only one out of four trials in which four sprays of 10 mgl^{-1} GA_{4+7} were applied and Elfving and Allen (1987) found return bloom was unaffected by three sprays of 20 mgl^{-1} or two sprays of 60 mgl^{-1} GA_{4+7} applied to Golden Delicious trees.

Eccher and Castelli (1982) demonstrated that the timing of the GA_{4+7} applications may also be important. When four sprays of 50 mgl^{-1} GA_{4+7} were applied to Golden Delicious trees starting from full-bloom up until three weeks after full-bloom, all treatments inhibited return bloom but the later the spray programmes were applied,the greater the effect. This result contrasts with that of Taylor (1978), who found no such relationship between timing of application and return bloom supression. It is interesting to note that Tromp (1982), who applied high rates (500 mgl^{-1}) of GA_{4+7} to potted trees of Cox Orange Pippin at various times, found that bud numbers were reduced on spurs at all timings, with the greatest effect from the early applications (full-bloom). In contrast the numbers of flower buds that developed on the current year's shoots were reduced more by later GA_{4+7} applications.

Comparing the relative effects of GA_3, GA_4 and GA_{4+7} on russeting in two apple cvs, Wertheim (1982) obtained an indication that GA_7 influenced return bloom more than GA_4. This was confirmed by Tromp (1982, 1987) who found a significant difference between the effects of the two gibberellins; GA_7 had a marked effect on return bloom whereas GA_4 had little or no effect. Indeed, it has been reported recently that GA_4 may even promote return bloom under certain conditions (Looney et al, 1985). Thus the evidence that exogenous GAs suppress return flowering is by no means unequivocal (see Pharis and King, 1985).

Gibberellin applications during flowering or immediately afterwards have either increased, decreased or had no effect on fruit set of apple (Dennis, 1986). Reductions in set have been recorded especially where high concentrations of GA_{4+7} have been applied (Taylor, 1978), but where lower, more commercially acceptable concentrations were used fruit set was reduced in some trials but not in others (Scholtens and Bootsma, 1981; Wertheim, 1982; Steenkamp et al, 1984; Elfving and Allen, 1987). A slight thinning effect of GA_{4+7} sprays on Golden Delicious fruit was recorded by Wertheim (1986a), who found that the effect was increased where NAAm and/or carbaryl were also applied, the combined effect being greater than when the chemicals were applied alone. This could be important in practical situations where NAAm or carbaryl are applied as thinning agents, together with GA_{4+7} as a russet control agent, although excessive thinning was only recorded where all three chemicals were applied.

The reduction in fruit set following GA_{4+7} applications might reflect a detrimental effect on seed number of fruit. Reductions in the number of seeds per fruit were recorded after high concentrations

of GA_{4+7} were applied (Taylor, 1975; Boffelli and Eccher, 1978; Eccher and Boffelli, 1981; Elfving and Allen 1987) and this effect is due to an increase in seed abortion (Varga, 1969; Wertheim, 1973).

Wertheim (1971) reported that high concentrations ($\geqslant$100 mgl^{-1}) of GA_{4+7} enhanced fruit size of Cox's Orange Pippin. At lower concentrations ($\leqslant$10 mgl^{-1}) Wertheim (1982) detected a slight increase in fruit size of Karmijn de Sonnaville, as did Cobianchi and Bagnara (1983) with Golden Delicious, and this enhancement of fruit size after gibberellin application has been recorded with other fruit species (Goodwin, 1978).

There are many reports of the effect of applied gibberellins on the shape of apple fruits, primarily an increase in the length/diameter (L/D) ratio of the fruit (See Dennis, 1986). Experimental evidence suggests that applications at full-bloom have more effect than either pre or post-bloom treatments (Stembridge and Morrell, 1972). Similar effects have been reported when high concentrations of GA_{4+7} have been applied to Jonathan (Taylor, 1975) and Golden Delicious (Boffelli and Eccher, 1978; Eccher and Boffelli, 1981; Eccher, 1983; Elfving and Allen, 1987). In some cases, an apparent increase in L/D ratio of fruit during the early part of the growing season was noted, this effect having disappeared by harvest (Taylor, 1975; Wertheim, 1982).

Effects of gibberellins on fruit shape are due to increased growth of the fruit tissues at the calyx end (Westwood and Bjornstad, 1968), a finding supported by the demonstration that localised applications of gibberellin to the stalk end of apple fruit had no effect on the L/D ratio (Westwood, 1978). Localised applications of GA_4 have increased growth of apple fruit tissues due to an increase in cell division and

size (Bukovac and Nakagawa, 1968; Nakagawa et al, 1968).

Although it is known that foliar applications of GA_3 can stimulate shoot growth in apple (Dennis and Edgerton, 1966; Luckwill, 1968), there are relatively few reports of a similar effect with GA_{4+7}. Wertheim (1973) reported that applications of 100 mgl^{-1} GA_{4+7} at various times after full-bloom stimulated shoot growth but the results were variable and any effects were small. Similarly Taylor (1978) found that shoot growth increased after sprays of GA_{4+7} at concentrations of 100 mgl^{-1} or above, but that lower concentrations had little effect. Using potted trees of Cox's Orange Pippin, Tromp (1982) recorded slight increases in growth after applications of 500 mgl^{-1} GA_3, GA_4, GA_7 or GA_{4+7} at full bloom and two or four weeks later.

The observed effects of exogenous gibberellins on shoot growth in apple can be related to the fact that it is known, at least in some plants, that internode extension is dependant upon endogenous gibberellins (Phinney, 1984; Ingram and Macmulan, 1986), and Koshioka et al (1985), using modern physico-chemical methods of analysis, have recently provided unequivocal evidence of the presense of endogenous gibberellins in the vegetative tissues of apple. The major gibberellin identified was GA_{19} with smaller amounts of GA_{20}, GA_1 and GA_9.

1.5.2.2.3. Gibberellin and Cytokinin Mixtures

The effects of gibberellin and cytokinin mixtures on the incidence of russeting in Golden Delicious has been reported by several workers. This work has usually involved the use of a commercial product containing equal proportions of GA_{4+7} and 6-benzyladenine (BA) (Promalin). Applications of Promalin have given significant reductions in the incidence of russeting of Golden Delicious fruit when applied as

multiple sprays in the immediate post-bloom period (Eccher, 1983; Steenkamp et al, 1984; Eccher and Maffi, 1986). Eccher (1983) compared the effect of GA_{4+7} + BA and GA_{4+7} alone and found there was little difference between the treatments in the reduction of russeting obtained. Steenkamp et al (1984) also obtained a similar result but the levels of GA_{4+7} in the GA_{4+7} + BA treatments were not equivalent to those of the GA_{4+7} alone, making firm conclusions impossible.

Trials have also taken place in which BA and GA_{4+7} as separate treatments have been applied. Taylor (1975) found that applications of BA significantly increased russeting on Jonathan fruit while GA_{4+7} significantly decreased it, but did not report results of a combined treatment. The effects of separate and combined treatments were reported, however, by McLaughlin and Greene (1984), who applied treatments of 50 mgl^{-1} BA and 25 mgl^{-1} GA_{4+7} to trees of Golden Delicious. The BA alone significantly increased russeting, GA_{4+7} alone significantly decreased russeting, while the combined treatment had no effect compared to untreated controls. These results suggest that where GA_{4+7} + BA has reduced russeting, GA_{4+7} is the active component.

A possible advantage in the use of GA_{4+7} + BA is that it may result in less flower bud inhibition, as shown by McLaughlin and Greene (1984). The application of BA alone was found to increase return bloom, while GA_{4+7} reduced it and the combined treatment produced no significant effect. In this work, the concentration of BA was double that of GA_{4+7}. Promalin reduced return bloom significantly although the effect was slightly less than that produced by GA_{4+7} alone (Eccher and Castelli, 1982).

1.5.2.2.4. Gibberellins and Other Compounds

Edgerton and Veinbrants (1979) found that GA_{4+7} and Apasil (silicon dioxide) gave the greatest reduction in russeting of Golden Delicious fruit. Similarly Steenkamp et al (1984), compared GA_{4+7} and Golclair and found the combined treatment to have the greatest effect. It seems probable that the mode of action of GA_{4+7} and the other compounds on russeting are different, resulting in an additive effect when combined.

1.5.2.2.5. Succinic Acid 2,2-dimethylhydrazide (daminozide)

Applications of the plant growth retardant daminozide have reduced the incidence of cracking in the fruit of susceptible cultivars. For example Costa et al (1983) found reduced fruit cracking of Stayman Red after spray applications of the product Alar during the growing season. Undesirable side-effects of such applications, such as a reduction in fruit size (Costa et al, 1983) and adverse effects on storage quality of some cultivars (Sharples and Johnson, 1986), have limited the commercial use of Alar for the control of cracking in apples.

1.6. PURPOSE OF RESEARCH

The aim of the work described in this thesis was three-fold. Firstly, it was considered important to identify those factors which could affect the efficacy of GA_{4+7} spray applications for russet control under UK commercial orchard conditions. The factors investigated included concentration, frequency and timing of application and their possible interactions. In addition, the potential synergistic effects of other plant growth regulators in combination with GA_{4+7} to reduce russeting and cracking, together with any possible side-effects, would be studied.

Secondly, to explore the effects of the application of GA_{4+7} and other plant growth regulators on the structure and physical characteristics of apple fruit skin, in order to gain some understanding of the mode of action of GA_{4+7} in the reduction of russeting, and to give further insight into the nature of the disorder.

Thirdly, to study the uptake and translocation of exogenous gibberellins in apple, in order to identify the target sites for GA_{4+7} spray applications and the implications for the control of russeting and cracking in commercial apple production.

PART I - APPLIED FIELD STUDIES

GENERAL MATERIALS AND METHODS

All experiments were conducted in orchards at East Malling Research Station, Maidstone, Kent, unless stated otherwise. Trees were planted in weed-free herbicide treated strips and maintained with a routine pest and disease control programme.

Sprays were applied to drip-point using a motorised high-pressure sprayer (RSM), with hand-held polythene or canvas screens being used to prevent spray drift reaching adjacent trees. Control trees were left unsprayed. Treatments were applied to single, whole trees and replicated within randomised block designs unless stated otherwise. Trees were assigned to blocks in each experimental layout, with blocks defined as containing trees with similar numbers of fruit* buds, counted before flowering. The numbers of fruit buds on each tree were counted, both before and in the year after treatment except where stated otherwise. Only spur and terminal buds were counted on the cultivars Cox's Orange Pippin and Discovery; buds on one year old wood were recorded on the cultivar Golden Delicious as a separate count.

Where fruit length/diameter ratios were determined, fifteen fruit per replicate were measured using calipers (Camlab Model C with dial, Camlab UK, Cambridge). Measurements of randomly selected fruit from all sides of the tree were taken during the season; selection was by choosing one fruit visually but then measuring the nearest adjacent fruit. At harvest, fruit was chosen randomly from boxes of harvested fruit.

* Name given to those buds which give rise to a cluster of flowers.

Usually fruit was picked on the appropriate commercial harvest dates, fruit from individual trees being placed into boxes. Fruit was either graded immediately or stored in cold, air or controlled atmosphere conditions and graded later. Harvested fruit were weighed and graded for size, russet and colour, unless stated otherwise.

Fruit storage experiments were conducted at the Fruit Storage Department of East Malling Research Station. Fruit stored in controlled atmospheres were loaded into half-ton capacity storage cabinets connected to an automatic atmosphere sampling and control system. The concentration (%) of soluble solids in the fruit was determined with a hand-held refractometer (Bellingham and Stanley), using juice extracted from the cortex of individual apples with a blunt-ended probe. Starch iodine staining patterns were assessed using a visual method based on that described for Conference pears by Cockburn and Sharples (1980). Fruit firmness (kg) was measured on two peeled, opposite sides of each fruit with a motorised penetrometer fitted with a 8 mm diameter convex probe, as described by Topping (1981).

Grading Standards

Fruit was sorted into four grades; these are described below for each cultivar and also illustrated in Plates 1 - 3.

Cox's Orange Pippin

Grade I fruit smooth with only a slight trace of russet in the stalk cavity.

Grade II up to 1/10 surface area with russet.

Grade III up to 1/3 surface area with russet or 1/20 with rough russet/small cracks.

Grade IV	over 1/3 surface area with russet or 1/20 with rough russet or cracks.

Discovery

Grade I	fruit smooth with only a slight trace of russet in the stalk cavity.
Grade II	up to 1/5 surface area with russet or 1/20 with rough russet/small cracks.
Grade III	up to 1/2 surface area with russet or 1/3 with rough russet/small cracks.
Grade IV	over 1/2 surface area with russet or 1/3 with rough russet/cracks.

Golden Delicious

Grade I	fruit completely smooth.
Grade II	russet in the stalk cavity and slight trace on cheek.
Grade III	up to 1/5 surface area with russet.
Grade IV	over 1/5 surface area with russet or any rough russet.

Grading for colour conformed to the standards specified in the EEC regulations (MAFF, 1973), unless stated otherwise.

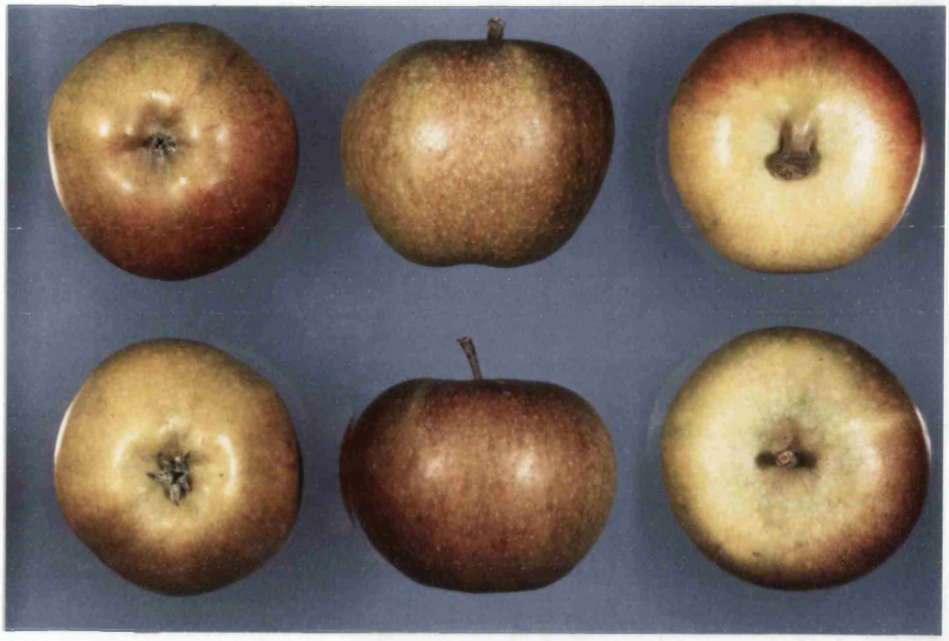
For the determination of the mineral composition of the fruit after harvest, two longtitudinal sections from opposite sides of the fruit were cut from each apple and any seeds removed. The combined sections from each sample were then finely chopped and weighed, and analysed for N, P, K, Mg and Ca content by the procedure described by Samuelson and Holland (1983). After the samples were digested in Kjeldahl mixture, N and P were measured colormetrically, K by flame emission spectroscopy, Ca by fluorimetry and Mg by atomic absorption

spectroscopy and the results expressed as mg $100g^{-1}$ fresh weight.

All data were analysed by analysis of variance and the standard error of difference (SED) presented where appropriate. For those experiments where the treatments were successive concentration of GA_{4+7} (Chapter 2), the 'concentrations' sum of squares in the analysis of variance table was partitioned into linear, quadratic and residual components, to provide information on the pattern of response.

Plate 1 - Russeting and cracking grading standards used for Cox's Orange Pippin.

(i) Grade I.

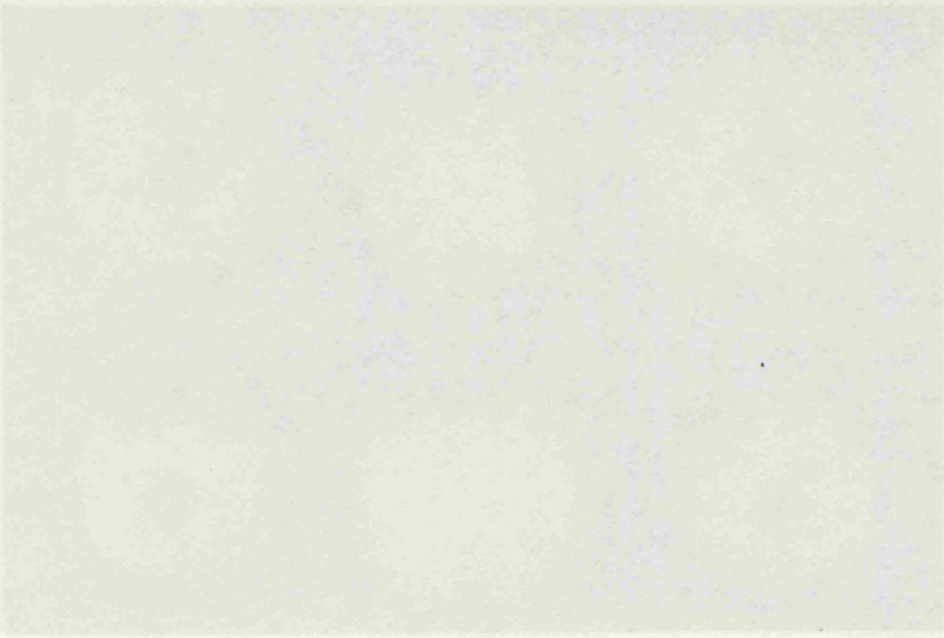


(ii) Grade II.

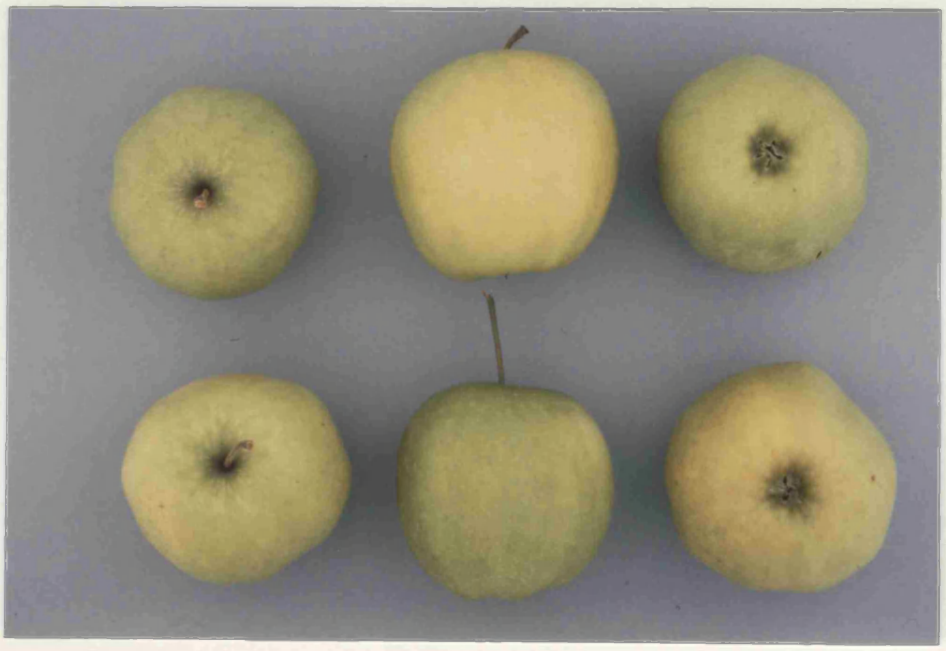
(iii) Grade III.

(iv) Grade IV.

Plate 2 - Russeting and cracking grading standards used for Golden Delicious.



(i) Grade I.



(ii) Grade II.

(iii) Grade III.

(iv) Grade IV.

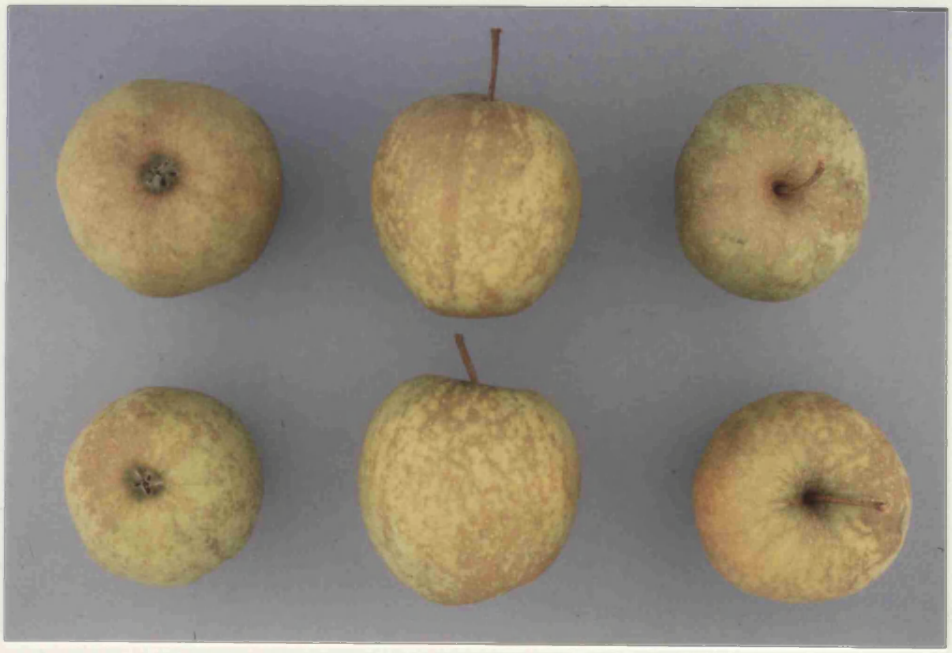
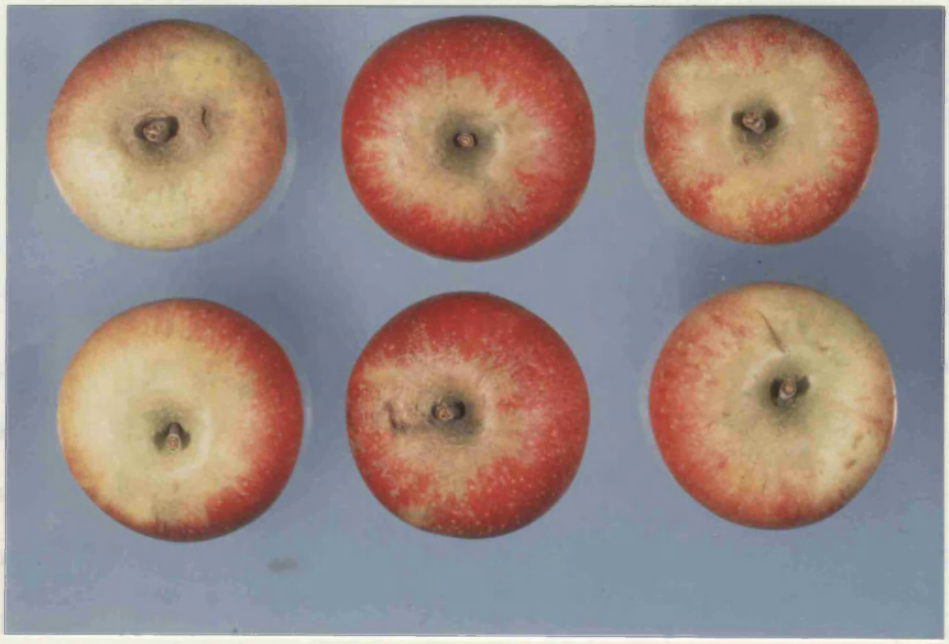


Plate 3 - Russet and cracking grading standards used for Discovery.

(i) Grade I.

(ii) Grade II.

(iii) Grade III.



(iv) Grade IV.

CHAPTER 2:

THE EFFECT OF CONCENTRATION OF GIBBERELINS A_4+A_7 ON THE INCIDENCE OF RUSSET AND CRACKING IN FRUIT OF APPLE CVS COX'S ORANGE PIPPIN, GOLDEN DELICIOUS AND DISCOVERY

2.1. INTRODUCTION

It is clear from results of trials conducted in various countries that spray applications of the gibberellins $A_4 + A_7$ (GA_{4+7}) immediately after flowering can give significant reductions in the incidence of russet and cracking of apple fruit (Taylor, 1975, 1978; Eccher, 1978; Eccher and Boffelli, 1978, 1981; Wertheim, 1980). Comparative trials with other compounds known to reduce russeting have shown GA_{4+7} to be the most effective treatment (Wertheim, 1980) and has proved to be active in trials involving several apple cultivars (Taylor, 1975; Scholtens and Bootsma, 1981; Joosse, 1982).

Although increasing concentrations have often shown an increased level of control of russet and cracking, these higher doses have produced detrimental side-effects, and would not provide cost effective spray programmes for use in the apple growing industry (Taylor, 1975, 1978; Scholtens and Bootsma, 1981). Recommendations for the commercial use of GA_{4+7} in Holland, based on early trials results, suggest the use of four sprays at ten day intervals, starting at petal-fall, with concentrations of 2.5-10 mgl^{-1} GA_{4+7}, depending on the cultivar (Scholtens and Bootsma, 1981; Westerlaken, 1982).

This chapter evaluates the influence of GA_{4+7} concentration on the incidence of russeting and cracking in three apple cultivars under UK growing conditions; side-effects of these treatments were assessed and their potential importance evaluated.

2.2. MATERIALS AND METHODS

Six experiments were conducted in 1983 and 1984 to evaluate the efficacy of a range of concentrations of GA_{4+7} with the cultivars Cox's Orange Pippin, Golden Delicious and Discovery. Details of the orchards, spraying dates and concentrations of GA_{4+7} applied are given in Table 2. In all experiments four sprays were applied at ten day intervals, the first at 80% petal-fall of flowers on two year or older wood.

TABLE 2

Experimental Details

Cultivar and year of treatment	Tree age yrs	Root stock	Planting distance m	Concentration * GA_{4+7} mgl^{-1}	Spraying dates
Cox's Orange Pippin 1983	7	M9	5.0 x 3.0	1.75, 3.5 7.0, 14, 28	20/5, 31/5 9/6, 20/6
Golden Delicious 1983	9	M9	4.1 x 2.5	1.75, 3.5 7.0, 14, 28	23/5, 2/6 13/6, 22/6
Discovery 1983	6	M26	4.8 x 2.4	1.75, 3.5 7.0, 14, 28	23/5, 2/6 13/6, 22/6
Cox's Orange Pippin 1984	17	M26	4.8 x 4.2	2.5, 5, 10 20	21/5, 31/5 11/6, 20/6
Discovery 1984 (1)	14	M26	4.5 x 2.3	5, 10, 20	18/5, 29/5 7/6, 18/6
Discovery 1984 (2)	9	MM106	4.5 x 3.0	5, 10	21/5, 31/5 11/6, 20/6

* - Concentrations given for 1983 are approximate due to errors in measuring water volumes in the spray tank (wrong dip-stick supplied). Intended concentrations were - 2.5, 5, 10, 20 and 40 mgl^{-1}.

1983

The experiment on Discovery was carried out at a grower farm in Kent. Treatments were replicated eight times in all experiments. During the growing season the length/diameter ratios of the fruit were determined, fifteen fruit per replicate being chosen at random (120 fruit per treatment). Fruit was harvested on the following dates: Discovery, 18 August; Cox's Orange Pippin, 20 September; Golden Delicious, 25-28 October. The Discovery fruit was brought to East Malling Research Station for grading. The Cox's Orange Pippin fruit was graded for russet and colour, the other cultivars being graded for russet only.

After grading the length/diameter ratios of the fruit were determined, as was the pedicel length of the Golden Delicious. Harvested fruit was cut open transversely and the number of seeds counted into two categories, fully developed (plump) and aborted (flat). After these measurements were completed the fruit samples were used for determination of mineral composition, except in the case of Discovery.

In order to examine possible effects on the maturity of Discovery fruits samples were taken one day before harvest (17 August), ten fruit being chosen at random from all sides of the tree, two replicates only of each treatment being sampled. The maturity was assessed by determination of internal ethylene concentration, soluble solids and starch-iodine staining patterns.

Internal ethylene concentration was determined as soon as possible after the fruit was picked (within four hours), using the method described by Knee et al (1983). From each apple, 0.5 ml of the

internal gas was drawn into a syringe through a hypodermic needle inserted into the core cavity. Gas samples were injected into a column (50 x 0.4 cm) of activated alumina (80-100 mesh) in a Pye 104 series gas chromatograph fitted with a flame ionization detector. This was calibrated with standards prepared volumetrically from pure ethylene which were injected before and after each sample and an average taken.

Storage assessments of Cox's Orange Pippin and Golden Delicious fruit involved samples from the control, 7 and 28 mgl^{-1} GA_{4+7} treatments. At harvest time twenty sound fruit were taken at random from all sides of the tree. Each sample was placed in a polypropylene net bag, weighed and dipped in a 0.05% solution of benomyl (Benlate). The samples were placed in metal boxes and put into a store on 21 September and 26 October respectively. Cox's Orange Pippin fruit was stored at 2% ±0.1% O_2 and <1% CO_2 at 3.5°C ±0.2°C, Golden Delicious being stored in air at 1.5°C ±0.2°C.

After removal from store on 27 March and 27 April 1984 respectively, the fruit was weighed and weight loss calculated. Wastage due to physiological disorders and fungal spoilage was assessed visually after cutting the fruit transversly. A sample of ten fruit of Cox's Orange Pippin and all the Golden Delicious fruit were used for fruit firmness determinations.

The remaining fruit of Cox's Orange Pippin were placed in wooden trays and tested for shelf-life by storage at 10°C for a further seven days. The fruit was then removed and examined for physiological disorders as before.

1984

Three experiments were conducted in 1984. In the Cox's Orange Pippin and the first Discovery (1) experiments, treatments were replicated eight times. The second Discovery (2) experiment involved a split-plot design with four blocks; the main plot treatments being three harvest dates, these being divided into three sub-plots, each comprising a single tree, for the three spraying dates.

Fruit in two blocks (2 and 4) of the Discovery (2) experiment was hand-thinned during 8-13 June, all clusters on the trees being reduced to a maximum of two fruit/cluster; lack of available labour prevented all blocks being thinned at this time. Initial fruit-set was recorded in the Cox's Orange Pippin and Discovery (1) experiments, the total numbers of fruit on whole trees being counted on 19-20 and 25-27 June respectively.

Fruit was harvested on the following dates: Discovery (1), 20-21 August; Discovery (2), 15, 22 and 29 August; Cox's Orange Pippin, 24-25 September. The fruit from Discovery (1) was graded for russet only; fruit from the first harvest of Discovery (2) was graded for russet and colour. Due to the lack of russet on any fruit from this harvest, fruit harvested on the remaining dates was graded for colour only. The Cox's Orange Pippin fruit was graded for russet and colour.

The grading standards used for colour varied with picking date of the Discovery (2) experiment; fruit from the first pick was sorted into four grades, fruit from the other picks being sorted into three, as follows:

Grade	15 August	22-29 August
I	<1/10 Surface area red	<1/3 Surface area red
II	1/10-1/3 Surface area red	1/3-1/2 Surface area red
III	1/3-1/2 Surface area red	>1/2 Surface area red
IV	>1/2 Surface area red	

After grading, ten Cox's Orange Pippin fruit per replicate were chosen at random and the length/diameter ratios determined. These samples were then used for mineral analysis.

Fruit from the Discovery (2) experiment was assessed for maturity, samples being taken from the three picks; 55 fruits were selected at random from all sides of the tree, from each of the four trees per treatment at each pick (220 fruits per treatment per pick). Ten fruits from each sample were taken for measurement of internal ethylene concentration, fruit firmness, soluble solids and starch iodine staining patterns as described earlier. Red colour development of the fruit epidermis was assessed visually scoring on a scale of 0 - 5 (no red colouration to total surface red) as used by Sharples and Little (1970).

Of the remaining 40 fruits in each sample, two sub-samples of 20 were each placed in a wooden tray and maintained at a temperature of 18^{o}C. One sub-sample was removed after three days, the other after six days, in order to simulate shelf-life conditions; the fruits were assessed for firmness, then cut transversely for an assessment of physiological disorders as described earlier.

The remaining fruits (5) of each sample were assessed for organaleptic qualities by a sensory panel.

Fruit from the Cox's Orange Pippin experiment were assessed for storage quality; from each tree 40 fruit were selected at random from all sites of the tree on 23 September and divided into two sub-samples of 20. These were placed into bags, weighed and dipped in a 0.05% solution of benomyl as described earlier. The fruits were stored at 3.5°C ±0.2C in each of the two storage atmospheres: 2% ±0.1% O_2, <1% CO_2 and 3% ±0.1% O_2, 5% ±0.2% CO_2 . Both batches of fruit were placed into store on 26 September, and removed on 19 March and 27 March 1985 respectively.

After removal from store the fruit from 2% O_2, <1% CO_2 was treated as described earlier, except that the shelf-life was assessed at 18°C for seven days. Fruit from 3% O_2 5% CO_2, was treated similarly together with an assessment for core flush; if present this was assessed visually using a rating of slight, moderate or severe. This rating was then used to calculate a core flush index for each sample as follows: $(n^1 \times 1) + (n^2 \times 2) + (n^3 \times 3)$, where n^1 = number of fruit with slight symptoms, n^2 = number of fruit with moderate, n^3 = number of fruit with severe. The maximum score for a sample of 20 fruit would be 60.

2.3. RESULTS

Cox's Orange Pippin

A thunderstorm on the 2nd August 1983 produced hailstones, up to 10mm in diameter, which caused considerable damage to the fruit; the skin being actually broken in many cases. Because of this, accurate grading for skin finish and colour was found to be impossible and no data is presented. Other data from this experiment is presented in Tables 3, 4 and 5.

The number of fruit harvested/100 blossom clusters was not reduced significantly by GA_{4+7} at 1.75 and 3.5 mgl^{-1}, but was reduced ($P<0.05$) by concentrations of 7.0 mgl^{-1} and above (Table 3). All concentrations, except 3.5 mgl^{-1}, reduced the number of fruit harvested per tree ($P<0.01$ for 28 mgl^{-1}, $P<0.05$ for others), with reductions of up to 30%. The mean fruit weight at harvest tended to be greater with GA_{4+7} treatment but this was only significant ($P<0.05$) in the case of the 1.75 mgl^{-1} treatment. The final yields per tree were also lower after GA_{4+7} treatment, significantly ($P<0.05$) in the case of the 14 and 28 mgl^{-1} treatments.

TABLE 3

Effect of various concentrations of GA_{4+7} applied in 1983 on the number of fruit harvested, mean fruit weight and final yield of Cox's Orange Pippin

GA_{4+7} concentration mgl^{-1}	Number of fruit harvested		Mean fruit weight g	Final yield /tree kg
	/100 clusters	/tree		
0.00	155.0	501	59.7	29.6
1.75	125.1	378	74.6	26.7
3.50	133.9	449	63.7	28.1
7.00	112.7	366	70.7	25.2
14.00	115.6	366	66.5	22.7
28.00	108.0	339	70.1	23.6
SED	17.3	50	6.3	2.9

SED = standard error of difference - 34 d.f. 1 missing value

The results of the GA_{4+7} treatments on fruit shape and seed number per fruit are given in Table 4. By July, GA_{4+7} treatments had

increased the length/diameter ratio of the fruit with the highly significant ($P<0.001$) linear effect of concentration. Although increased length/diameter ratios were also recorded at harvest only the 14 mgl^{-1} GA_{4+7} treatment gave rise to a significant ($P<0.05$) effect and no significant linear effect of GA_{4+7} concentration was detected. The number of seeds per fruit tended to be lower in the case of the GA_{4+7} treatments but this apparent effect was not significant. There were no significant treatment effects on the N, P, K, Mg or Ca concentrations of the fruit measured at harvest (data not presented). Data was not collected on the number of flower buds produced in 1984, as very few buds were produced on any of the trees.

TABLE 4

Effect of various concentrations of GA_{4+7} applied in 1983 on fruit shape during the season and at harvest and number of seeds per fruit of Cox's Orange Pippin

GA_{4+7} concentration mgl^{-1}	Length/diameter ratio		Number of seeds/ fruit
	11 July	Harvest	
0.00	0.881	0.825	6.3
1.75	0.906	0.844	5.9
3.50	0.896	0.839	6.4
7.00	0.914	0.842	5.9
14.00	0.919	0.850	5.8
28.00	0.924	0.843	5.7
SED	0.007	0.005	0.3

SED = standard error of difference (35 d.f.)

The effects of GA_{4+7} on the storage quality of Cox's Orange Pippin are shown in Table 5. Fruit treated with GA_{4+7} were less firm after

storage and had lost less water during storage than untreated fruit, which were also smaller, but none of these differences were significant. No significant effects of GA_{4+7} on the susceptibility of the fruit to physiological disorders developing during storage were detected.

TABLE 5

Effect of various concentrations of GA_{4+7} applied in 1983 on the storage quality of Cox's Orange Pippin fruit after a period of controlled atmosphere storage (2% O_2, <1% CO_2)

GA_{4+7} concentration mgl^{-1}	Mean fruit weight g	Fruit firmness kg	Weight loss %	Physiological disorders %
0.0	70.0	2.67	5.1	3.1
7.0	77.6	2.63	4.5	0.0
28.0	77.0	2.51	4.5	0.0
SED	5.5	0.13	0.3	-

SED = standard error of difference (14 d.f.)

The results of the experiment conducted in 1984 are given in Tables 6 and 7. All GA_{4+7} concentrations reduced the amount of russeting and cracking of the fruit, represented by the significant ($P<0.01$) increases in the proportion of fruit in russet grades I + II (Table 6). All concentrations produced an increase in grade I + II fruit, approaching 20%, with little difference between the concentrations. There were no treatment effects on fruit colour (data not presented).

No significant differences were found in the initial set/100 blossom clusters, number of fruit harvested/100 blossom clusters, number of fruit harvested/tree or final yield/tree. A significant ($P<0.05$)

increase in mean fruit weight was found only with the 2.5 mgl^{-1} GA_{4+7} application.

The smallest number of flower buds/tree was produced in 1985, after the highest concentration of GA_{4+7} had been applied, but no treatment effect was significant. There was no effect of GA_{4+7} on the length/diameter ratio of the fruit measured at harvest (data not presented).

TABLE 6

Effect of various concentrations of GA_{4+7} applied in 1984 on fruit quality, initial set, number of fruit harvested, mean fruit weight, final yield, and number of flower buds in 1985 of Cox's Orange Pippin

GA_{4+7} conc mgl^{-1}	Proportion fruit in russet grades I & II % wt	Initial set/ 100 clusters	Number of fruit harvested		Mean fruit weight g	Final yield /tree kg	Number of flower buds/ tree
			/100 clusters	/tree			
0.0	63.6	164.2	88.9	454	103.8	46.3	1162
2.5	82.0	140.7	93.3	488	117.7	55.9	1410
5.0	79.8	144.2	94.3	491	110.4	53.5	1407
10.0	81.4	148.7	93.2	456	115.4	52.5	1246
20.0	82.7	141.8	88.6	463	97.9	45.2	859
SED	4.7	19.7	11.2	63	6.7	7.2	214

SED = standard error of difference (28 d.f.)

Fruit was stored in two controlled atmosphere regimes after which fruit was assessed.

Storage in 2% O_2, <1% CO_2. Fruit treated with GA_{4+7} tended to be larger although no significant differences were detected (Table 7).

TABLE 7

Effect of various concentrations of GA_{4+7} applied in 1984 on the storage quality of Cox's Orange Pippin fruit after a period of storage in two controlled atmosphere conditions

GA_{4+7} concentration mgl^{-1}	2% O_2, <1% CO_2					3% O_2, 5% CO_2						
	Mean fruit Weight	Fruit firmness	Weight loss	Bitter pit ex store	Bitter pit after shelf -life	Mean fruit weight	Fruit firmness	Weight loss	Bitter pit ex store	Bitter pit after shelf -life	Core flush ex store	Core flush after shelf -life
	g	kg	%	%	%	g	kg	%	%	%		
0.0	110.2	2.34	5.3	5.0	6.9	107.5	2.42	5.7	6.2	7.1	5.0	7.0
2.5	121.1	2.32	5.1	8.7	12.9	116.6	2.35	5.6	1.2	11.2	11.2	15.1
5.0	116.9	2.32	4.7	10.0	23.3	111.0	2.35	5.0	3.7	6.7	6.2	9.0
10.0	118.5	2.21	5.5	8.7	15.8	117.9	2.15	5.6	2.5	5.3	20.0	12.6
20.0	109.1	2.26	4.6	3.7	12.6	102.6	2.27	4.8	1.2	7.5	8.7	1.2
SED	5.6	0.05	0.5	-	-	5.0	0.07	0.3	-	-	-	-

SED = standard error of difference (28 d.f.)

Fruit firmness was correlated negatively with GA_{4+7} concentration, with a significant ($P<0.05$) linear relationship being found. There was no clear influence of GA_{4+7} on weight loss during storage, all treatments giving similar effects.

There was an apparent increase in the incidence of bitter-pit in fruit from some GA_{4+7} treatments, both after storage and after a simulated shelf-life period, but there was no consistent trend with increasing GA_{4+7} concentration.

Storage in 3% O_2, 5% CO_2. Fruit treated with GA_{4+7} tended to be larger, a significant ($P<0.05$) increase being found with the 10 mgl^{-1} treatment but no linear relationship was detected. Fruit firmness was negatively correlated with GA_{4+7} concentration, with a significant ($P<0.01$) linear relationship being detected. Only 20 mgl^{-1} GA_{4+7} gave rise to a significant ($P<0.05$) reduction in weight loss during storage, compared with the untreated control. There was no clear trend in the effect of GA_{4+7} treatment on the incidence of bitter-pit and core flush, either after storage or after a simulated shelf-life period.

Golden Delicious

All GA_{4+7} treatments reduced the amount of russeting, as demonstrated by the increase in the proportion of fruit in russet grade I (Table 8). This effect was significant ($P<0.05$) with the 7 and 28 mgl^{-1} GA_{4+7} treatments, while all concentrations significantly increased the proportion of fruit in russet grades I + II. The proportion in russet grades I + II was increased by 10 to 15%, but although the greatest effect was found with the highest concentration, there was not a significant linear trend.

TABLE 8

Effect of various concentrations of GA_{4+7} applied in 1983 on fruit quality, number of fruit harvested, mean fruit weight and final yield of Golden Delicious

GA_{4+7} concentration mgl^{-1}	Proportion of fruit in russet grade I % wt	grade I+II % wt	Number of fruit harvested /100 clusters	/tree	Mean fruit weight g	Final yield /tree kg
0.00	4.1	74.9	164.7	534	79.9	44.5
1.75	7.1	85.1	153.3	501	85.3	44.6
3.50	9.8	87.1	135.1	435	87.9	39.7
7.00	11.7	89.5	143.5	475	84.1	42.5
14.00	9.1	88.9	141.5	426	92.1	41.9
28.00	13.5	90.7	149.5	447	93.2	44.1
SED	3.5	4.2	19.0	47	4.0	3.9

SED = standard error of difference (35 d.f.)

Although the numbers of fruit harvested/100 flower clusters were lower with all the GA_{4+7} treatments, none of these differences were significant, whereas significant ($P<0.05$) reductions in the number of fruit harvested/tree followed treatment with 3.5 and 14 mgl^{-1} GA_{4+7}. Mean fruit weight was increased by GA_{4+7}, with a significant ($P<0.05$) linear effect of increasing concentration. There was no treatment effect on final yield/tree.

The effect of the two highest GA_{4+7} concentrations on mean fruit weight was reflected in the marked decrease ($P<0.01$) in the proportions of fruit graded-out <55mm, and the corresponding increase ($P<0.001$) in the proportion graded-out >65 mm (Table 9). The proportion graded-out in the 55-60 mm size category was also reduced by the 14 and 28 mgl^{-1}

GA_{4+7} treatments ($P<0.05$ and $P<0.01$ respectively).

TABLE 9

Effect of various concentrations of GA_{4+7} applied in 1983 on the proportion of Golden Delicious fruits graded-out in different size categories

GA_{4+7} concentration mgl^{-1}	Size grades mm.			
	% <55	% 55-60	% 60-65	% >65
0.00	25.3	34.7	20.4	18.3
1.75	17.5	36.6	22.3	23.7
3.50	15.3	32.1	24.4	28.2
7.00	18.1	34.4	23.1	24.4
14.00	11.6	26.9	19.8	40.7
28.00	12.3	24.4	21.5	41.8
SED	4.6	3.5	2.8	6.1

SED = standard error of difference (35 d.f.)

By July, the GA_{4+7} treatments had increased the length/diameter ratio of the fruit, with a highly significant ($P<0.001$) linear effect of increasing concentration (Table 10). The effect, although less, was still measurable at harvest and a significant ($P<0.05$) linear effect of concentration was still apparent. The number of seeds per fruit tended to be lower where GA_{4+7} had been applied but the trend was not significant and there was no treatment effect on pedicel length. There were no significant treatment effects on the N, P, K or Mg concentrations of the fruit measured at harvest (data not presented). The figures for Ca were 6.92 (untreated), 6.96 (1.75 mgl^{-1}), 6.65 (3.5 mgl^{-1}), 6.39 (7.0 mgl^{-1}), 6.23 (14.0 mgl^{-1}) and 6.00 (28 mgl^{-1}) expressed as mg 100 g^{-1} fresh weight, with a significant ($P<0.001$)

linear effect of GA_{4+7} concentration (SED 0.20, 35 d.f.).

TABLE 10

Effect of various concentrations of GA_{4+7} applied in 1983 on fruit shape during the season and at harvest, number of seeds per fruit, length of pedicel and the number of flower buds in 1984 of Golden Delicious

GA_{4+7} concentration mgl^{-1}	Length/diameter ratio		Number of seeds/ fruit	Length of pedicel mm	Number of flower buds/tree
	12 July	Harvest			
0.00	1.061	0.996	6.0	27.3	173
1.75	1.105	1.007	6.0	27.4	146
3.50	1.125	1.022	5.4	26.8	194
7.00	1.131	1.023	5.6	28.4	88
14.00	1.131	1.022	5.8	27.7	133
28.00	1.134	1.031	5.2	26.6	160
SED	0.010	0.010	0.3	0.9	55

SED = standard error of difference (35 d.f.)

The number of flower buds produced per tree in 1984 were very variable, as a consequence of the biennial bearing habit of this cultivar, such that no significant treatment effects were found.

Results of the storage trial are given in Table 11. The fruit treated with 28 mgl^{-1} GA_{4+7} was significantly ($P<0.01$) larger than the untreated control fruit and were also significantly ($P<0.01$) softer and lost less water ($P<0.01$) in store. No physiological disorders occurred in treated or untreated fruit during storage.

TABLE 11

Effect of various concentrations of GA_{4+7} applied in 1983 on the storage quality of Golden Delicious fruit after a period of air storage

GA_{4+7} concentration mgl^{-1}	Mean fruit weight g	Fruit firmness kg	Weight loss %	Physiological disorders %
0.0	97.4	1.93	5.6	0.0
7.0	107.4	1.89	5.4	0.0
28.0	117.7	1.85	4.9	0.0
SED	6.8	0.02	0.2	-

SED = standard error of difference (14 d.f.)

Discovery

Results of the experiments conducted in 1983 are given in Tables 12, 13 and 14. GA_{4+7} had no effect on the incidence of russeting and cracking, the proportion of fruit in russet grades I + II being similar for all treatments (Table 12). There were no significant treatment effects on the number of fruit harvested/100 blossom clusters, number of fruit harvested/tree or the final yield/tree, although considerable variation occurred in the former two variables. The mean fruit weight was significantly ($P<0.05$) increased by the 28 mgl^{-1} GA_{4+7} treatment compared to the untreated control but there was no evidence of any effect with the lower concentrations.

TABLE 12

Effect of various concentrations of GA_{4+7} applied in 1983 on fruit quality, number of fruit harvested, mean fruit weight and final yield of Discovery

GA_{4+7} concentration mgl^{-1}	Proportion of fruit in russet grades I+II % wt	Number of fruit harvested /100 clusters	Number of fruit harvested /tree	Mean fruit weight g	Final yield /tree kg
0.00	76.7	81.6	507	73.8	36.9
1.75	76.2	78.0	466	77.7	36.6
3.50	83.8	86.7	542	81.5	43.4
7.00	73.9	71.7	424	81.6	34.2
14.00	81.2	80.3	507	78.8	38.8
28.00	80.7	68.5	425	90.2	38.3
SED	5.5	11.1	62	5.7	4.5

SED = standard error of difference - 25 d.f. 10 missing values - SEDs adjusted

The length/diameter ratios of GA_{4+7} treated fruits were increased by July, a significant ($P<0.01$) linear relationship with concentration being detected (Table 13). Although the effect had diminished by harvest time, a significant ($P<0.05$) linear effect could still be measured. The number of seeds tended to be lower in fruits treated with GA_{4+7} but there was not a consistent response.

When counted in 1984 the number of flower buds/tree tended to be lower on the trees treated with GA_{4+7} and the lowest number recorded was for trees sprayed with 28 mgl^{-1}. Results were very variable, however, and no significant treatment effects were measured.

TABLE 13

Effect of various concentrations of GA_{4+7} applied in 1983 on fruit shape during the season and at harvest, number of seeds per fruit and the number of flower buds in 1984 of Discovery

GA_{4+7} concentration mgl^{-1}	Length/diameter ratio		Number of seeds/ fruit	Number of flower buds/ tree
	28 July	Harvest		
0.00	0.760	0.757	6.5	413
1.75	0.769	0.762	6.0	409
3.50	0.783	0.767	5.6	425
7.00	0.779	0.765	6.2	333
14.00	0.784	0.771	5.2	303
28.00	0.793	0.773	5.6	300
SED	0.011	0.006	0.4	90

SED = standard error of difference - 30 d.f. 5 missing values - SEDs adjusted

Effects on maturity

Results of the initial experiment to determine possible effects of GA_{4+7} on the maturity of Discovery apples are given in Table 14. The results provide some evidence of an effect insofar as the fruit treated with GA_{4+7} had, on average, a higher internal ethylene concentration, together with a higher level of soluble solids and a lower level of starch, although there is no clear evidence of a concentration response.

TABLE 14

Effect of various concentrations of GA_{4+7} applied in 1983 on the internal ethylene level, soluble solids and starch content of Discovery apples at harvest

GA_{4+7} concentration mgl^{-1}	Internal ethylene - proportion of fruit with a level >0.1 ppm %	Soluble solids %	Starch %
0.00	66	8.9	94
1.75	80	10.1	77
3.50	80	9.4	86
7.00	90	9.3	92
14.00	100	10.2	85
28.00	70	9.3	91

Results from the two trials conducted in 1984 are given in Tables 15 to 18.

In the first trial, all GA_{4+7} treatments reduced the incidence of russeting and cracking, demonstrated by the significant ($P<0.05$) increase from 12 to 18%, in the proportion of fruit in russet grades I + II (Table 15). The effects of the various treatments were similar so that no significant linear effect of concentration could be detected.

The initial set/100 blossom clusters was found to be lower on trees treated with GA_{4+7} but the differences were not significant and no concentration response was detected. Although the number of fruits harvested/100 blossom clusters and the number of fruits harvested/tree were also lower where GA_{4+7} had been applied, these differences were again not significant, although a linear effect of GA_{4+7} concentration on the two variables just failed to reach a significant level.

TABLE 15

Effect of various concentrations of GA_{4+7} applied in 1984 on initial set, fruit quality, numbers of fruit harvested, mean fruit weight, final yield, and number of flower buds in 1985 of Discovery

GA4+7 conc. mgl-1	Proportion of fruit in russet grades I & II % wt	Initial set/ 100 clusters	Number of fruit harvested		Mean fruit weight g	Final yield /tree kg	Number of flower buds/ tree
			/100 clusters	/tree			
0.0	63.4	158.9	76.3	218	92.6	19.9	517
5.0	81.7	107.4	64.5	182	86.6	15.5	392
10.0	75.9	107.4	56.9	163	100.7	16.3	402
20.0	77.0	132.1	58.9	161	94.4	15.5	240
SED	4.6	25.2	9.7	30	5.9	2.8	47

SED = standard error of difference (21 d.f.)

No significant effects were found on the mean fruit weight or on the final yield/tree. The number of flower buds produced in 1985 were reduced by all GA_{4+7} treatments. The 5 and 10 mgl^{-1} treatments gave rise to reduced ($P<0.05$) numbers compared to the untreated control trees, while the 20 mgl^{-1} treatment gave rise to the lowest number of buds ($P<0.001$) compared to the control and ($P<0.01$) compared to the other GA_{4+7} treatments.

In the second experiment the results of the various agronomic factors are given in Table 16. No russeting or cracking was apparent in any of the fruit harvested in this experiment and hence no data is presented. GA_{4+7} treatment had no effect on fruit colour, number of fruit harvested/tree or final yield/tree. Mean fruit weight was increased significantly ($P<0.05$) by the GA_{4+7} treatments but no difference between the concentrations was detected.

The number of flower buds produced per tree in 1985 tended to be lower on those trees sprayed with GA_{4+7} the previous year but differences were not statistically significant.

TABLE 16

Effect of various concentrations of GA_{4+7} applied in 1984 on the red colour, number of fruit harvested, mean fruit weight, final yield and number of flower buds in 1985 of Discovery

GA_{4+7} conc. mgl^{-1}	Colour-proportion of fruit with >1/2 surface area red %	Number of fruit harvested /tree	Mean fruit weight g	Final yield /tree kg	Number of flower buds/tree in 1985
0.0	58.1	561	68.0	38.1	807
5.0	63.8	527	74.6	38.7	719
10.0	61.3	527	73.9	38.2	613
SED	3.4	31	2.6	2.5	101

SED = standard error of difference (18 d.f.). Figures relate to the results from three harvest dates combined

Effect on maturity

The levels of internal ethylene in fruit were not affected by GA_{4+7} treatment irrespective of harvest date (Table 17). No effect on fruit firmness was detected with fruit harvested on 15 and 22 August, whereas fruit harvested on 29 August was significantly ($P<0.001$) softer from trees treated with GA_{4+7}. The red colouration of fruit harvested on 15 and 29 August was unaffected, whereas fruit harvested on 22 August was significantly ($P<0.05$) redder as a result of GA_{4+7} treatment.

TABLE 17

Effect of various concentrations of GA_{4+7} applied in 1984 on maturity indices of Discovery apples harvested on three different dates

GA_{4+7} Concentration mgl^{-1}	Internal ethylene - proportion of fruit with a level >0.1ppm %			Fruit firmness kg			Red colouration 0-5			Soluble solids %			Starch %		
Harvest	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0.0	54	92	100	3.94	3.51	3.09	2.8	3.0	3.9	9.7	10.1	10.9	83	57	15
5.0	65	85	100	3.79	3.53	2.62	2.8	3.5	4.2	10.2	10.2	11.8	79	54	11
10.0	49	77	100	3.83	3.49	2.72	2.8	3.6	3.9	9.9	10.5	11.1	78	47	11
SED between harvests	-	-	-		0.25			0.3			0.4			7	
SED between treatments	-	-	-		0.07			0.2			0.3			6	

SED = standard error of difference. Harvest versus Harvest - 6 d.f. Treatment versus Treatment - 18 d.f.

Harvest 1 - 15 August 1984; Harvest 2 - 22 August 1984; Harvest 3 - 29 August 1984

GA_{4+7} did not affect the levels of soluble solids or starch in fruit significantly at any harvest date. Sensory analysis of fruit harvested on the three dates showed no consistent treatment effects (data not presented).

Fruit firmness, measured after three or six days of shelf-life treatment, was consistently lower in GA_{4+7} treated fruit, irrespective of harvest date (Table 18). The differences were only significant, however, in the fruit treated with 10 mgl^{-1} GA_{4+7}, harvested on 22 and 29 August, after three days of shelf-life treatment ($P<0.05$).

TABLE 18:

Effect of various concentrations of GA_{4+7} applied in 1984 on the firmness (kg) of Discovery apples harvested on three different dates, after two shelf-life treatments

	Shelf-life 1 3 days at 18°C			Shelf-life 2 6 days at 18°C		
Harvest	1	2	3	1	2	3
GA4+7 conc. mgl-1						
0.00	3.71	3.54	3.14	2.45	2.60	2.45
5.00	3.61	3.39	2.87	2.26	2.50	2.14
10.00	3.54	3.10	2.77	2.38	2.44	2.22
SED between harvests		0.17			0.17	
SED between treatments		0.16			0.17	

SED = standard error of difference.

Harvest versus harvest - 6 d.f. Treatment versus treatment - 18 d.f.

Harvest 1 - 15 August 1984; Harvest 2 - 22 August 1984;

Harvest 3 - 29 August 1984

2.4. DISCUSSION

The results reported here clearly demonstrate that several applications of GA_{4+7} in the period immediately after flowering, can significantly improve the skin finish of Cox's Orange Pippin, Golden Delicious and Discovery fruit under UK growing conditions, corresponding with results reported elsewhere from other countries (Taylor, 1975, 1978; Eccher, 1978, 1983; Eccher and Boffelli, 1978; Edgerton and Veinbrants, 1979; Wertheim, 1980, 1982; Joosse, 1982; Westerlaken, 1982). Indeed, in only one trial did GA_{4+7} treatments fail to improve skin finish, and this was where low ambient levels of russet and cracking were present. It is apparent that the efficacy of the treatments increased where there was a high incidence of the disorders.

Although no significant effects of GA_{4+7} concentration were found with any of the cultivars, there was some evidence of a positive relationship between skin finish and concentration with Golden Delicious. Such a relationship has been shown in some trials (Taylor, 1975; Elfving and Allen, 1987) whereas in others (Eccher, 1978, Eccher and Boffelli, 1978, 1981), results have been inconsistent with either little or a variable response being detected. The lack of any effect of concentration with Cox's Orange Pippin has also been reported from Holland (Westerlaken, 1982), in contrast to results with the cultivar Karmijn de Sonnaville where increased concentrations of GA_{4+7} have given consistently better control of russet and cracking (Scholtens and Bootsma, 1981; Westerlaken, 1982). These apparent inter-cultivar differences in response to GA_{4+7} concentration in relation to russet control cannot be explained at present but could be due to a number of factors. There may be differences in the patterns of uptake and

movement of exogenous gibberellins between cultivars and/or in the rates of metabolism within the plant tissues. In addition the effects may be related to variable tissue sensitivity to applied gibberellins (see Firn, 1986).

The thinning action of GA_{4+7} applications, especially with the higher concentrations used, which was apparent in many of the trials has been recorded by other workers (Taylor, 1978; Scholtens and Bootsma, 1981; Wertheim, 1982, 1986a; Steenkamp et al, 1984). This is in contrast to the effects of GA_{4+7} applied to emasculated or frost damaged flowers, where treatment usually results in an increase in the numbers of fruit retained to harvest (Dennis and Edgerton, 1966; Wertheim, 1971, 1973). The thinning action after application to open-pollinated flowers is a variable response, however, and has been attributed to either increased shoot/fruit competition due to enhanced shoot growth, or a decrease in the seed number resulting in a reduction in the competitive vigour of the fruitlets (Wertheim, 1982).

Some reduction in the seed numbers of fruit as a result of GA_{4+7} treatment was apparent in this work and elsewhere (Taylor, 1975; Eccher and Boffelli, 1981; McLaughlin and Greene, 1984, Elfving and Allen, 1987) and has been related to an increase in seed abortion (Wertheim, 1973). Similarly a reduction in the number of seeds per fruit and fruit set after GA_{4+7} applications at blossom time has been widely reported in many grape cultivars (eg Weaver and Pool, 1971). Interestingly Greene (1984) found that direct applications of GA_{4+7} + BA to flowers of Early McIntosh apple led to a reduced number of seeds but did not effect fruit set, leading to the suggestion that the fruit thinning effect of gibberellins when applied to whole trees is related

to subtle effects on shoot growth and carbon partitioning (Looney and Pharis, 1986). Application of high concentrations of GA_{4+7} can result in increased shoot growth of apple (Taylor, 1978; Tromp, 1982) but the effect is minimal at lower concentrations (Wertheim, 1973).

The increases in fruit size that were recorded were undoubtably due in many cases to the fruit thinning effect of GA_{4+7}, but direct effects on size were also apparent, especially with Discovery. Increased fruit size caused by GA_{4+7} has been reported previously, both in the case of apple (Wertheim, 1971, 1982) and in many other fruit species (see Goodwin, 1978). Localised applications of high concentrations of GA_4 were found to increase the growth of apple fruit tissues by increasing both cell number and size (Bukovac and Nakagawa, 1968; Nakagawa et al, 1968), and similar effects were recorded where gibberellin increased berry size of seedless grape (Sachs and Weaver, 1968). It seems likely that consistent increases in fruit size will occur only with high concentrations of GA_{4+7}, whereas those being examined for russet control (<10 mgl^{-1}) result only in small and inconsistent reponses.

It is apparent that treatment with GA_{4+7} altered the fruit shape of all three cultivars in this study causing the fruit to be more elongated, the magnitude of the effect varying with concentration of GA_{4+7} applied, the stage of fruit development and the cultivar (Figure 1). This is a well documented effect of such treatments, both in apple (Dennis, 1986) and other fruit species (Stembridge, 1973). The present findings show, as in earlier reports, that the effect is most marked early in fruit development and diminishes as the fruit develops, (Taylor, 1975; Wertheim, 1982), corresponding with the normal change in

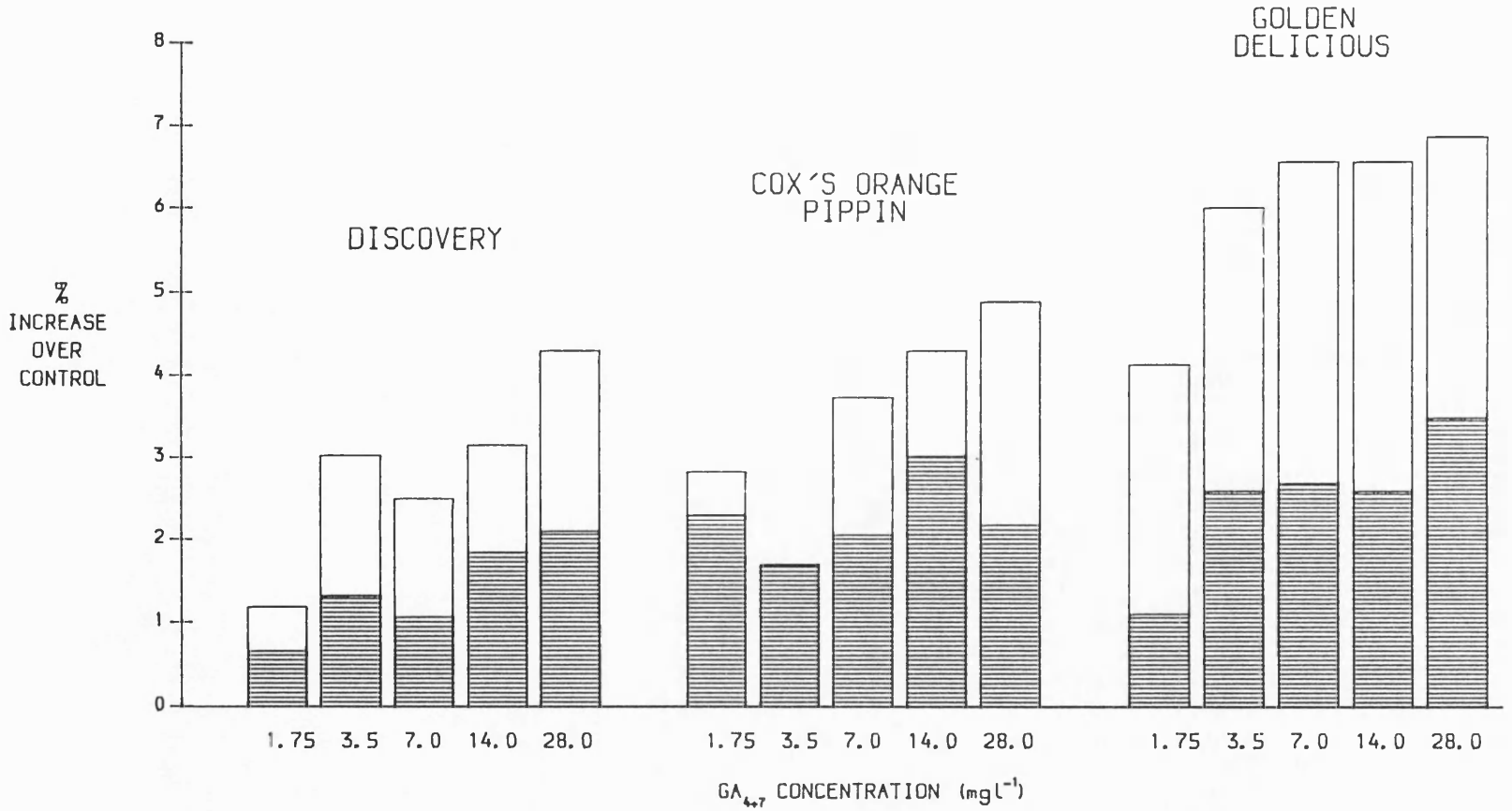


Fig. 1. Increase in the length/diameter ratio of fruit from trees of three cultivars sprayed with various concentrations of GA_{4+7} in 1983, measured in July ☐ and at harvest ▤

fruit shape whereby the length/diameter ratio is at its greatest soon after flowering and diminishes as the season progresses (Westwood, 1962). The effects on shape were most marked in Golden Delicious which of the three cultivars typically has the longest fruit and in which fruit shape has been related to the endogenous gibberellin content of the fruit (Eccher, 1986).

It is probable that fruit shape is determined by the balance of endogenous hormones with gibberellins playing a major role, the process being mediated via an effect on the direction of cell enlargement rather than enlargement per se (Westwood, 1978), and that exogenous GA alters the balance in favour of elongation. Support for the pivotal role of endogenous gibberellins in determining fruit shape comes from the results of work in which paclobutrazol, a potent inhibitor of gibberellin biosynthesis, reduced the length/diameter ratio of apple fruit, the effect being reversed by exogenous gibberellin (Currey and Williams, 1983).

Although few significant effects of GA_{4+7} on return bloom were recorded in this study it is still clear that treatment, especially with high concentrations, could reduce the number of flower buds produced the following year. This was true especially in the case of Discovery where a negative relationship between the number of flower buds and GA_{4+7} concentration was detected, as has been found with Golden Delicious in other studies (Taylor, 1978; Eccher and Castelli, 1982; Meador and Taylor, 1987). It is clear, however, that applications of GA_{4+7} at low concentrations (≤ 10 mgl^{-1}) generally have little or no effect on return bloom, as shown here and elsewhere (Eccher and Castelli, 1982; Wertheim, 1982; Elfving and Allen, 1987;

Meador and Taylor, 1987). Since, as has been mentioned earlier, low concentrations of GA_{4+7} give optimum control of russet and cracking in Cox's Orange Pippin, the commercial application of such treatments should not involve any serious effect on flower bud production.

Although GA sprays applied close to full bloom have been reported to enhance the ripening of apples under certain conditions (Sharples and Johnson, 1986), there was little evidence of such an effect when applied to Discovery in this work. The effects of GA_{4+7} on fruit firmness which were recorded in some cases may be due to a direct effect, perhaps on the cell walls of the fruit or may be related to the confounding effect on fruit size. Increased fruit size may lead to reduced Ca content of the fruit as measured with Golden Delicious, as there is an inverse relationship between calcium concentration and fruit size (Perring, 1979). Although low Ca levels have been associated with increased risk of physiological disorders developing during storage, especially bitter pit (Perring, 1986), there was no evidence of any adverse effect of GA_{4+7} on the storage quality of the fruit in this study or elsewhere (Wertheim, 1982). Indeed GA_{4+7} treatments may have positive effects by reducing the water lost during storage due to the improved skin finish of the fruit (Lott, 1957). Again, there seems to be little risk of any deleterious side efects of GA_{4+7} applied at low concentrations to apples for russet control.

CHAPTER 3:

THE EFFECTS OF NUMBER OF SPRAYS, SPRAY INTERVAL AND TIME OF APPLICATION OF GIBBERELLINS $A_4 + A_7$ ON THE INCIDENCE OF RUSSET AND CRACKING IN FRUIT OF APPLE CV COX'S ORANGE PIPPIN

3.1. INTRODUCTION

There is considerable evidence that the period immediately after flowering is critical for the initiation of russeting and that this period is when control measures against russet must be applied. In Golden Delicious petal-fall was found to be the time of greatest sensitivity to copper sprays that caused russeting (Bondoux et al, 1971), and control measures, whether physical protection by paper bags (Creasy and Swartz, 1981) or spray applications of anti-russet compounds such as dimethoate (Skene, 1980b), were found to give the greatest reduction in russeting when applied at this time. Sprays of GA_{4+7} at petal-fall were found to be more effective than later applications (Eccher, 1978; Eccher and Boffelli, 1978, 1981; Taylor, 1978).

Although in general multiple applications of GA_{4+7} have given a greater reduction of russet (Eccher and Boffelli, 1978, 1981; Taylor, 1978; Scholtens and Bootsma, 1981), other evidence suggests that the period of time over which the spray programme is applied can influence the results (Westerlaken, 1982). Also, Eccher and Boffelli (1981) considered that the combination of optimum timing and number of spray applications was more important for the control of russeting in Golden Delicious with GA_{4+7}, than its concentration in the spray.

The experiments in this chapter are concerned with the relative effects of timing, number of sprays and spray interval, together with

the dose of GA_{4+7} supplied on the control of russet and cracking of Cox's Orange Pippin under UK growing conditions.

3.2. MATERIALS AND METHODS

Two experiments were conducted on Cox's Orange Pippin in order to evaluate the effects of timing, spray interval and number of applications of GA_{4+7}.

1983

GA_{4+7} at a concentration of *7 mgl^{-1} was applied via a range of spray programmes in which time of initial application, number of sprays and the spray interval were varied (Table 19). The various programme components were combined factorially to give a total of thirty-six treatments, plus untreated controls. Because of the large number of treatments, replicates were based at two sites: three replicates at East Malling Research Station and one at a growers farm in Kent. Orchard details are given in Table 20, actual spraying dates being given in Appendix I. Controls were replicated four times at both sites.

Fruit was harvested on the following dates - East Malling, 26-28 September; Horsmonden, 19 September. All fruit was graded at East Malling Research Station. After grading, samples of fifteen fruit per treatment were selected at random from the three replicates at East Malling for determination of the mineral content.

*Approximate figures due to errors in measuring water volume in the spray taken (wrong dip-stick supplied). Intended concentration 10 mgl^{-1}.

TABLE 19

Spray programme variables

Timing of initial application	Number of spray applications	Spray interval days
First Flower (king flowers open)	2	7
Full bloom	4	14
80% petal fall of flowers on 2 year or older wood	6	
7 Days after T3		
14 Days after T3		
21 Days after T3		

TABLE 20

Orchard details

Orchard	Tree age yrs	Rootstock	Planting distance m
East Malling	18	M26	5.0 X 4.2
Horsmonden	6	MM106	6.4 X 5.5

1984

The experiment was conducted on eleven year old trees on MM106 rootstock at a planting distance of 5.0 m x 4.2 m. Time of initial application, number of spray applications and spray interval were all varied (Table 21); GA_{4+7} concentration was also varied in order to equalise the total amounts of GA_{4+7} applied in each treatment. Treatments were replicated eight times.

Fruit was harvested on 25 September and graded. At harvest twenty fruit per replicate from treatments 1, 4 and 7 were taken at random, from all sides of the tree, to test for effects on storage potential. The fruit was stored from 26 September in a controlled atmosphere of 2% ± 0.1% O_2 and <1% CO_2 at 3.5°C ±0.2°C , the fruit being assessed as as for the Cox fruit from the 1984 concentration experiment, (see Chapter 2), on 26 March 1985.

TABLE 21

Treatment details and spraying dates

Treatment number	Time of first applications	No. of sprays	Spray conc. mgl^{-1}	Spray interval days	Spraying dates
1	Control				
2	Green Cluster	4	10.0	10	1/5,11/5,21/5,31/5
3		2	20.0	30	11/5,11/6
4	First flower	4	10.0	10	11/5,21/5,31/5,11/6
5		6	6.7	6	11/5,17/5,23/5,29/5 4/6,11/6
6		2	20.0	30	23/5,22/6
7	Petal-fall	4	10.0	10	23/5,31/5,12/6,22/6
8		6	6.7	6	23/5,29/5,4/6,11/6 15/6,22/6

First flower - all king flowers open

Petal-fall - 80% petal-fall of flowers on two year or older wood

3.3. RESULTS

The skin finish of fruit from this experiment was generally

excellent, all treatments producing approximately 88% of fruit in russet grades I + II (data not presented). The proportion of fruit in russet grade I only is presented (Table 22). Two trends can be identified; firstly that spray programmes starting at the earlier dates, especially those starting at first flower, reduced the incidence of russeting and cracking to a greater extent than spray programmes starting at later dates, with the least control being achieved by programmes starting twenty-one days after petal-fall. Secondly, spray programmes with four or six spray applications reduced the incidence of russeting and cracking to a greater extent than those involving only two spray applications. When the results were averaged for the different variables, it was found that there was no significant difference between spray programmes involving seven or fourteen day spray intervals.

Table 23 gives the results of the proportion of fruit in russet grade I combined for spray interval and the general trends noted above are again apparent. Spray programmes involving four or six spray applications produced significantly ($P<0.001$) more fruit in russet grade I than programmes involving two spray applications starting at first flower, the six spray-programme being also significantly better ($P<0.05$) than the two-spray programme at the full bloom timing. The proportion of fruit in russet grade I produced by the six-spray programme starting at first flower was significantly ($P<0.05$) greater than the majority of the other treatments and was highly significant ($P<0.001$) when compared to the control. All treatments produced significantly ($P<0.05$) more fruit in russet grade I when compared to the control, except for the two-spray programmes starting at first flower and full bloom.

TABLE 22.

The effect of GA_{4+7} applied in different combinations of number of sprays, starting time and spray interval in 1983 on fruit quality of Cox's Orange Pippin, presented as percentage weight of fruit in russet grade I

No. of sprays	Spray interval days	Time of first spray: First flower	Full bloom	Petal-fall	+7 days	+14 days	+21 days
Control	7.4						
2	7	10.8	8.6	10.2	11.2	13.7	11.4
2	14	9.8	12.4	12.8	19.1	11.1	11.6
4	7	21.5	14.9	11.9	13.9	14.4	12.1
4	14	15.3	13.3	13.8	11.5	14.4	9.5
6	7	18.8	13.5	13.3	16.5	12.1	14.0
6	14	19.3	17.1	11.8	12.1	12.8	11.5
SED treatments versus control				2.6			
SED between treatments				3.3			

SED = standard error of difference (120 d.f.)

Combined results from two sites - East Malling and Horsmonden - (four replicates)

TABLE 23.

The effect of GA_{4+7} applied in different combinations of number of sprays and starting time in 1983 on fruit quality of Cox's Orange Pippin, presented as percentage weight of fruit in russet grade I

	Time of first spray					
No. of sprays	First flower	Full bloom	Petal-fall	+7 days	+14 days	+21 days
Control	7.4					
2	10.3	10.5	11.5	15.1	12.4	11.5
4	18.4	14.1	12.8	12.7	14.4	10.8
6	19.1	15.3	12.5	14.3	12.4	12.7
SED treatments versus control			2.0			
SED between treatments			2.4			

SED = standard error of difference (120 d.f.)

Combined results from two sites - East Malling and Horsmonden - (four replicates)

When the results are combined over start-time, spray programmes involving four or six spray applications produce significantly ($P<0.05$) more fruit in russet grade I than programmes involving two spray applications; 13.9% and 14.4% compared to 11.9%. If the results are combined over number of sprays, spray programmes starting at first flower produce significantly ($P<0.05$, $P<0.05$, $P<0.01$ respectively) more fruit in russet grade I than programmes starting at petal-fall, petal fall plus fourteen days and petal-fall plus twenty-one days; 15.9% compared to 12.3%, 13.1% and 11.7% respectively.

No treatment effects were apparent on the number of fruit harvested/100 bloom clusters, number of fruit harvested/tree, mean fruit weight or final yield/tree (data not presented). There were no treatment effects on the N, P, K, Mg or Ca composition of the fruit determined at harvest (data not presented).

The number of flower buds produced in 1984 varied considerably from tree to tree, this variability being reflected in the experimental data presented in Table 24. No significant differences between the treatments could be detected, although most trees treated with GA_{4+7} produced fewer buds than the controls. When the results are combined over spray number, trees treated with spray programmes involving four or six spray applications produced significantly fewer ($P<0.05$) buds than the control trees; 117 and 116 buds/tree compared to 239 buds/tree. The results when combined over spray interval show that trees treated with spray programmes involving a seven day spray interval produced fewer ($P<0.05$) flower buds than control trees; 129 compared to 239 buds/tree but there was no significant effect of fourteen day spray programmes. No significant effect of different start-times on the production of flower buds could be detected.

TABLE 24.

The effect of GA_{4+7} applied in different combinations of number of sprays, starting time and spray interval in 1983 on the number of flower buds per tree of Cox's Orange Pippin in 1984

No. of sprays	Spray interval days	Time of first spray: First flower	Full bloom	Petal-fall	+7 days	+14 days	+21 days
Control		239					
2	7	226	243	132	212	134	115
2	14	295	372	189	101	145	104
4	7	95	251	63	28	80	79
4	14	178	128	102	66	224	107
6	7	196	55	125	24	48	212
6	14	56	86	200	199	95	98
SED				132			

SED = standard error of difference (120 d.f.)

Results from one site - East Malling (three replicates)

1984

As in 1983 the skin finish of the fruit was excellent, with all treatments producing between 85% to 90% of the fruit in russet grades I + II (Table 25) and no significant treatment effects were detected. No differences were found when only the fruit in russet grade I was considered (data not presented).

TABLE 25

The effect of GA_{4+7} applied in different combinations of number of sprays, starting time and spray interval in 1984 on fruit quality of Cox's Orange Pippin, presented as percentage weight of fruit in russet grades I + II

Number of sprays		Time of first spray: Green cluster	First flower	Petal-fall
Control	88.7			
4		86.1		
2			86.7	91.3
4			91.0	90.5
6			86.9	90.9
SED			3.6	

SED = standard error of difference (49 d.f.)

No significant treatment effects on the number of fruit harvested/100 blossom clusters, number of fruit harvested/tree, mean fruit weight or final yield/tree were recorded (data not presented).

There were no significant treatment effects on the number of flower buds produced in 1985 (Table 26).

TABLE 26

The effect of GA_{4+7} applied in different combinations of number of sprays, starting time and spray interval in 1984 on the number of flower buds per tree in 1985 of Cox's Orange Pippin

Number of sprays		Time of first spray		
		Green cluster	First flower	Petal-fall
Control	684			
4		632		
2			768	662
4			578	474
6			645	655
SED			132	

SED = standard error of difference (49 d.f.)

There was no significant treatment effect on fruit firmness, weight loss or the incidence of physiological disorders in fruit after a period of controlled atmosphere storage (Table 27). No effect of GA_{4+7} treatment was detected on the incidence of physiological disorders in fruit from storage that had been given a shelf-life treatment (data not presented).

TABLE 27

The effect of GA_{4+7} applied in spray programmes starting at the beginning or end of flowering in 1984 on the storage quality of Cox's Orange Pippin

Treatment	Mean fruit weight g	Fruit firmness kg	Weight loss %	Physiological disorders %
Control	94.1	2.26	4.0	0
GA_{4+7} (BB)	97.7	2.26	3.6	0
GA_{4+7} (EB)	96.9	2.27	3.6	0
SED	6.1	0.05	0.3	-

SED = standard error of difference (14 d.f.)

BB - four sprays at ten day intervals starting at first flower

EB - four sprays at ten day intervals starting at petal-fall

Stored in 2% O_2, <1% CO_2 at 3.5°C

3.4. DISCUSSION

Despite the generally good skin finish and thus small treatment effects in both years, there was a clear indication from the 1983 experiment that GA_{4+7} spray programmes must commence in the flowering period to give the greatest improvement in skin finish, with the effect diminishing as treatment is delayed. This corresponds with the findings of other workers (Taylor, 1978; Eccher and Boffelli, 1981; Eccher, 1983) and points to the flowering period being the critical time during which russet can be initiated and when control measures should be applied. Further support for this critical period for russet initiation and control comes from work with other techniques to reduce

the incidence of russeting, such as covering the fruit with paper bags (Creasy and Swartz, 1981).

The evidence that variation in the timing of the first spray application within the flowering period might result in differences in the level of russet control is less clear cut. Although this work suggests that spray programmes commencing at the beginning of flowering are the most effective, other work has shown no consistent response when the timing of the first spray is varied within this period (Westerlaken, 1982; Eccher and Boffelli, 1981; van Rooijen, 1983; Elfving and Allen, 1987). Possibly the optimum timing of the first GA_{4+7} application within the flowering period will vary from year to year and from site to site, depending on the causal factors responsible for russet initiation in each particular situation.

It is clear that multiple spray programmes are more effective than those involving a low number of applications, although this may reflect the different amounts of GA_{4+7} applied in total rather than the number of sprays per se, as in the 1983 experiment. Similar observations have been reported from other studies involving up to eight repeat applications (Eccher and Boffelli, 1978; Scholtens and Bootsma, 1981; Elfving and Allen, 1987). Interestingly there was little difference between spray programmes involving four or six applications in this work, which may be related to the time period over which the spray programmes were applied. As stated previously, it is the flowering period which is critical for the initiation of russet and this is when control measures should be applied; hence the later sprays in a multiple spray programmes could be expected to have little or no effect. Indeed Eccher and Boffelli (1981) found a significant negative

correlation between the duration of the GA_{4+7} spray programme and the incidence of russeting.

Although the 1984 experiment was designed to evaluate the effect of multiple spray applications, while taking into account the potentially confounding effects of both the amount of GA_{4+7} applied and the length of time involved, the results were disappointing due to the good skin finish of the fruit. Similar experiments conducted in Holland have shown that spray programmes involving six GA_{4+7} sprays can be more effective than those involving four sprays (Dijke and Kester, 1983), where the same amount of GA_{4+7} is applied within the same period of time. This could be related to the multiple sprays maintaining a higher level of exogenous gibberellins within the plant tissues. Gibberellin A_4 is known to be rapidly metabolised in apple (Looney *et al*, 1978), although it is probable that the metabolism of GA_7 is less rapid (Pharis and King, 1985). Metabolism of the applied GA_{4+7} to inactive or less active metabolites would continuously deplete the levels in the plant tissues and it is feasible that the effect of such depletion could be minimised by multiple GA_{4+7} applications, thus leading to more effective control of russeting.

The timing of GA_{4+7} treatments had no detectable effect on the degree of inhibition of return bloom in the 1983 experiment, probably due to the low numbers of floral buds produced generally in the spring of 1984 masking any treatment effects. Other studies have shown an effect of timing; thus Eccher and Castelli (1982) demonstrated that GA_{4+7} spray programmes commencing at full-bloom had the least effect on return bloom of Golden Delicious, the degree of inhibition increasing as the first spray timing was delayed up to three weeks after

full-bloom. Taylor (1978), however, found no such difference with similar spray programmes, the reduction in return bloom detected being equal, irrespective of timing.

Interestingly, Tromp (1982) found with Cox's Orange Pippin that the effect of time of application of GA_{4+7} on return bloom depended on the age of wood on which the flower buds were produced. The number of flower buds formed on spurs were reduced by a full-bloom spray more than later applications, while the reverse was true for buds formed on new wood. It was suggested in the latter case that little shoot growth had taken place by the time of the full-bloom application and consequently the GA_{4+7} would have little effect at this time, in contrast to the later applications.

From the practical viewpoint of potential commercial applications of GA_{4+7} for russet control it should be stressed that, as the 1984 experiment demonstrated, the use of low concentrations of GA_{4+7} will have little or no effect on return bloom irrespective of the time of application.

CHAPTER 4:

THE EFFECTS OF GIBBERELLINS ALONE AND IN COMBINATION WITH OTHER PLANT GROWTH REGULATORS ON THE INCIDENCE OF RUSSET AND CRACKING IN FRUIT OF APPLE CVS COX'S ORANGE PIPPIN AND GOLDEN DELICIOUS

4.1. INTRODUCTION

Both gibberellins GA_3 and a mixture of GA_4 and GA_7 have been shown to reduce russet and cracking if applied several times in the post-bloom period, although GA_3 is the least efficacious (Eccher, 1978; Eccher and Boffelli, 1978; Taylor, 1978; Wertheim, 1982). Further work by Wertheim (1982), has shown slight differences in efficacy between the gibberellins GA_4 and GA_7, with GA_4 appearing to be slightly more effective than GA_7.

Other plant growth regulators have also been shown to have effects on the incidence of apple fruit russeting. Auxin-like substances have reduced russeting in some cases, eg NAAm (Schumacher and Fankhauser, 1967; Schumacher et al, 1977) and 2,4,5-TP (Byers et al, 1983), whereas cytokinins have increased the incidence of russeting (Eccher, 1975; Taylor, 1975; McLaughlin and Greene, 1984). It is possible that combinations of plant growth regulators may give more effective control of russeting than GA_{4+7} alone, as has been suggested in trials in which GA_{4+7} was combined with other anti-russeting compounds (Edgerton and Veinbrants, 1979; Steenkamp et al, 1984).

The experiments described in this chapter evaluate the effects of GA_3, GA_4, GA_7 and GA_{4+7}, both alone and in combination with auxins and cytokinins, on the incidence of russeting in two apple cultivars.

4.2. MATERIALS AND METHODS

Four experiments were conducted on two cultivars, Cox's Orange Pippin and Golden Delicious. In all cases four sprays of each treatment were applied at ten day intervals, the first at approximately 80% petal-fall of the flowers on two year or older wood, all treatments being replicated eight times.

1983

Two experiments were conducted in 1983, one on each cultivar. Orchard details and spraying dates are given in Table 28, a list of treatments in Table 29.

TABLE 28

Orchard details and spraying dates

Cultivar	Tree age yrs	Root stock	Planting distance m	Spraying dates
Cox's Orange Pippin	17	M26	5.0 x 4.2	23/5,2/6,13/6,22/6
Golden Delicious	15	M26	4.8 x 4.2	23/5,3/6,13/6,22/6

The GA_3 was prepared from the proprietary formulation Berelex (ICI Agrochemicals, Horticulture). Gibberellins A_4 and A_7 were not available in a pure form and were prepared, therefore, by separating the components of a GA_{4+7} mixture (crystalline technical grade, supplied by ICI). Separation was achieved using a high performance liquid chromatography (HPLC) system.

TABLE 29

Details of treatments

Treatment		Concentration *mgl^{-1}
1	Control	-
2	GA_3	7
3	GA_4	7
4	GA_7	7
5	GA_{4+7}	7
6	GA_{4+7} + IAA	7 + 7
7	GA_{4+7} + NAA	7 + 7
8	GA_{4+7} + BA	7 + 7

*Concentrations given are approximate due to errors in measuring water volumes in the spray tank (wrong dip-stick supplied). Intended concentration 10 mgl^{-1}

All treatments were applied to Cox's Orange Pippin, treatments 3 and 4 being omitted on Golden Delicious.

The technique involved the use of a Lichrosorb RP8 column (20 x 1 cm with 10µm packing), eluted with acidified methanol (50% v/v + 50 μl^{-1} acetic acid), at a constant flow rate of 3 cm^3 min^{-1} using a Pye Unicam LC3-XP pump. Absorbance was measured at 215 nm using a Cecil Instrument CE212 ultra violet monitor linked to a Cecil chart recorder. A fraction collector (model FRAC-100, Pharmacia Fine Chemicals), was coupled in series directly after the monitor to facilitate collection of the relevant aliquote. A 100µl sample of GA_{4+7} (1 g in 10 cm^3 30% methanol) was loaded onto the column on each occasion.

In order that samples could be loaded automatically, a Magnus Scientific M7100 autosampler was placed in series before the column, which enabled samples to be loaded at ~ 30 min intervals. The

autosampler was also linked to the fraction collector such that a pre-programmed collection procedure started when a sample was loaded. Retention times of GA_4 and GA_7 were of the order of 20 and 22 min respectively. The system allowed for 22 samples to be loaded and collected automatically before needing to be reset.

Samples of GA_4 and GA_7 were bulked-up and acidified below pH 3.0 with 0.1 M hydrochloric acid. This was then partitioned against equal volumes of ethyl acetate, the aqueous phase being discarded. The ethyl acetate fraction was then reduced to dryness under vacuum in a rotary evaporator, the resulting crystalline gibberellins being weighed and stored at $-18^{o}C$ prior to formulation. Purities of the gibberellins obtained were found to be 100% for GA_7 and over 97% for GA_4.

Solvents used throughout were either HPLC grade or were freshly distilled.

The GA_4 and GA_7 were formulated by dissolving in propylene glycol (BP grade - Brenntag UK Ltd, Kingston upon Thames, UK), to make a concentration of 10 gl^{-1}.

Indolyl-3-acetic acid (IAA) was prepared from crystalline IAA (96% ai, BDH Chemicals Ltd, Poole, UK), by dissolution in a small quantity of methyl alcohol prior to dilution in water. The 1-naphthylacetic acid (NAA) was the commercial formulation Planofix (May and Baker Chemical Company Ltd), and the GA_{4+7} + 6-benzyladenine (BA) mixture was the commercial formulation Promalin (Abbot Laboratory, North Chicago, USA), containing equal quantities of GA_{4+7} and BA.

During the season, the length/diameter ratio of 15 fruit/replicate from both experiments was determined. Cox's Orange Pippin was

harvested on 26 - 28 September, Golden Delicious on 26 - 28 October. At harvest 20 fruit were taken from each replicate in the Golden Delicious experiment, to assess treatment effects on storage potential; the fruit was stored in air at 1.5°C ±0.2°C until 27 April 1984 and assessed as described previously for Golden Delicious (see Chapter 2, 1983 experiment).

After grading the length/diameter ratios of the fruit were again determined and the length of the pedicel was measured, again on 15 fruit per replicate. Each fruit was then cut open transversly and the seeds counted into two categories: fully developed (plump) or aborted (flat).

1984

Two experiments were conducted in 1984, one on each cultivar. Orchard details and spraying dates are given in Table 30, a list of treatments in Table 31.

TABLE 30

Orchard details and spraying dates

Cultivar	Tree age yrs	Root stock	Planting distance m	Spraying dates
Cox's Orange Pippin	11	MM106	5.0 x 4.2	23/5,2/6,13/6,22/6
Golden Delicious	16	M26	4.8 x 4.2	25/5,4/6,15/6,25/6

TABLE 31

Details of treatments

Treatment		Concentration mgl^{-1}	
		Cox's Orange Pippin	Golden Delicious
1	Control	-	-
2	GA_{4+7}	10	5
3	Enriched GA_{4+7}	10	5
4	GA_{4+7} + NAA (1)	10 + 2.5	5 + 1
5	GA_{4+7} + NAA (2)	10 + 5.0	5 + 2.5
6	GA_{4+7} + BA	10 + 10	5 + 5

The enriched GA_{4+7} formulation (JF 4834 - ICI), contained a high proportion of GA_4 compared to the normal formulation Regulex, consisting of 8.36 gl^{-1} GA_4 + 2.45 gl^{-1} GA_7.

Cox's Orange Pippin was harvested on 25 September, Golden Delicious on 15 October.

4.3. RESULTS

Cox's Orange Pippin

1983

Skin finish of the fruit in this trial was, generally, excellent with 85% or more in russet grades I + II in all cases (Table 32) and no significant treatment effects were observed. If the proportion of fruit in russet grade I is considered, however, the GA_4, GA_{4+7} and GA_{4+7} + NAA improved ($P<0.05$) skin finish compared to the control, while GA_{4+7} + BA produced a highly significant ($P<0.001$) effect almost doubling the proportion of fruit in russet grade I compared to the control.

No significant treatment effects were observed on the number of fruit harvested/100 blossom clusters, number of fruit harvested/tree, mean fruit weight or final yield/tree.

TABLE 32

Effect of gibberellins, alone and in combination with other plant growth regulators applied in 1983 on fruit quality, number of fruit harvested, mean fruit weight and final yield of Cox's Orange Pippin

Treatment	Proportion of fruit in russet		Number of fruit harvested		Mean fruit weight	Final yield /tree
	grade I % wt	grade I+II % wt	/100 clusters	/tree	g	kg
Control	9.5	84.6	130.3	509	90.5	45.3
GA_3	13.4	88.4	126.0	477	97.0	46.0
GA_4	15.2	88.1	127.5	512	90.0	45.4
GA_7	13.8	88.5	132.7	518	87.2	45.4
GA_{4+7}	14.3	88.9	120.9	462	95.1	43.3
GA_{4+7} + IAA	14.0	88.4	125.8	465	95.1	44.2
GA_{4+7} + NAA	15.4	86.1	129.9	505	83.7	43.3
GA_{4+7} + BA	18.2	90.2	123.1	480	92.5	43.3
SED	2.4	2.7	15.0	53	5.3	5.6

SED = standard error of difference (49 d.f.)

By July all treatments had increased the length/diameter ratio of the fruit significantly ($\underline{P}<0.001$, all except GA_{4+7} + NAA, $\underline{P}<0.01$) (Table 33), although by harvest only fruit treated with GA_4, GA_7, GA_{4+7} + IAA and GA_{4+7} + BA treatments were still showing a significant ($\underline{P}<0.01$ for GA_4, GA_{4+7} + BA; $\underline{P}<0.05$ for GA_7, GA_{4+7} + IAA) elongation when compared to the control value.

The number of full seeds per fruit was unaffected by any

treatment, except for GA_{4+7} + NAA which caused a reduction ($P<0.05$) compared to the untreated control.

Numbers of flower buds produced in 1984 varied considerably from tree to tree and no significant treatment effects were detected.

TABLE 33

Effect of gibberellins, alone and in combination with other plant growth regulators applied in 1983 on fruit shape during the season and at harvest, number of seeds per fruit and the number of flower buds in 1984 of Cox's Orange Pippin

Treatment	Length/diameter ratio		Number of seeds/ fruit	Number of flower buds/tree
	18 July	Harvest		
Control	0.854	0.835	5.5	421
GA_3	0.877	0.838	5.6	420
GA_4	0.886	0.854	5.0	421
GA_7	0.889	0.850	5.3	345
GA_{4+7}	0.902	0.845	5.7	258
GA_{4+7} + IAA	0.883	0.852	5.0	547
GA_{4+7} + NAA	0.869	0.837	4.6	266
GA_{4+7} + BA	0.911	0.859	5.4	305
SED	0.005	0.007	0.4	144

SED = standard error of difference (49 d.f.)

1984

Skin finish of the fruit was also excellent, with nearly all treatments producing about 90% in russet grades I + II (Table 34) and no treatment effects were detected.

An apparent thinning action of the enriched GA_{4+7} treatment was detected, as shown by the significant ($P<0.05$) reduction in both

number of fruit harvested/100 blossom clusters and number harvested/ tree. No other treatment effects on numbers of fruit were detected. Mean fruit weight was significantly ($P<0.05$) increased by the enriched GA_{4+7}, GA_{4+7} + NAA (1) and the GA_{4+7} + BA treatments when compared to the control, although there was no treatment effect on the final yield/tree. No treatment effects were found on the number of flower buds produced in 1985.

TABLE 34

Effect of gibberellins GA_{4+7}, alone and in combination with other plant growth regulators applied in 1984 on fruit quality, number of fruit harvested, mean fruit weight, final yield and number of flower buds in 1985 of Cox's Orange Pippin

Treatment	Proportion of fruit in russet grade I + II % wt	Number of fruit harvested /100 clusters	Number of fruit harvested /tree	Mean fruit weight g	Final yield /tree kg	Number of flower buds/tree
Control	88.4	109.1	473	83.0	38.8	773
GA_{4+7}	91.8	85.2	391	102.1	37.0	591
Enriched GA_{4+7}	85.2	73.7	329	111.0	33.5	714
GA_{4+7} + NAA(1)	90.3	79.2	353	110.3	37.0	708
GA_{4+7} + NAA(2)	89.7	85.8	381	101.1	39.2	913
GA_{4+7} + BA	90.5	82.9	356	110.0	37.9	888
SED	2.7	15.9	70	10.3	5.9	135

SED = standard error of difference (35 d.f.)

NAA(1) - 2.5 mgl^{-1}, NAA(2) - 5.0 mgl^{-1}

Golden Delicious

1983

All treatments reduced the incidence of russeting as shown by

increases in the proportions of fruit in russet grades I and I + II (Table 35). GA_{4+7} and GA_{4+7} + NAA increased the proportion of fruit in grade I to the greatest degree compared to the control. All treatments increased significantly ($\underline{P}<0.001$ except GA_3, $\underline{P}<0.05$) the proportion of fruit in russet grades I + II when compared to the control, with increases ranging from 5 to 10%.

A thinning effect by both the GA_3 and GA_{4+7} + NAA treatments was detected. GA_3 reduced ($\underline{P}<0.05$) both the number of fruit harvested/100 blossom clusters and number harvested/tree compared to the control, with the GA_{4+7} + NAA treatment causing a highly significant ($\underline{P}<0.001$) reduction in both parameters. These two treatments reduced ($\underline{P}<0.05$ and $\underline{P}<0.001$) the final yield/tree and the GA_{4+7} + NAA also caused a significant ($\underline{P}<0.001$) increase in fruit size. No other treatment effects on fruit number, size or final yield were detected.

The thinning action of the GA_{4+7} + NAA resulted in a more than 40% reduction ($\underline{P}<0.05$) in the proportion of fruits graded-out under 60mm in diameter, more than 60% reduction in the proportion graded-out in the 60-65mm size category ($\underline{P}<0.001$), and an increase of over 180% ($\underline{P}<0.001$) in the proportion graded out over 70mm in diameter.

TABLE 35

Effect of gibberellins, alone and in combination with other plant growth regulators applied in 1983 on fruit quality, number of fruit harvested, mean fruit weight and final yield of Golden Delicious

Treatment	Proportion of fruit in russet		Number of fruit harvested		Mean fruit weight	Final yield /tree
	grade I % wt	grade I+II % wt	/100 clusters	/tree	g	kg
Control	8.6	86.6	153.0	760	113.2	85.8
GA_3	10.7	91.3	106.0	592	114.7	67.1
GA_{4+7}	17.6	96.1	127.0	669	112.9	75.8
GA_{4+7} + IAA	14.4	95.0	131.4	701	111.3	76.8
GA_{4+7} + NAA	24.6	96.3	81.8	445	123.1	54.8
GA_{4+7} + BA	12.2	94.3	124.9	685	112.8	76.7
SED	-	2.0	17.7	73	2.7	7.8

SED = standard error of difference (35 d.f.)

- SED not valid

TABLE 36

Effect of gibberellins, alone and in combination with other plant growth regulators applied in 1983 on the proportion of Golden Delicious fruits graded-out in different size categories

Treatment	Size grades mm.			
	% <60	% 60-65	% 65-70	% >70
Control	11.4	15.0	61.8	11.8
GA_3	11.7	11.2	62.4	14.7
GA_{4+7}	12.0	12.3	62.1	13.5
GA_{4+7} + IAA	15.1	12.7	57.4	14.7
GA_{4+7} + NAA	6.5	5.6	53.7	34.2
GA_{4+7} + BA	13.8	11.1	57.4	17.7
SED	2.2	2.4	2.9	3.9

SED = standard error of difference (35 d.f.)

All treatments except GA_3 increased ($P<0.001$) the length/diameter ratio of the fruit, both by July and at harvest (Table 37).

The number of full seeds per fruit measured at harvest was reduced by GA_{4+7} + IAA ($P<0.05$), GA_{4+7} + NAA and GA_{4+7} + BA (both $P<0.001$). No effect was detected on fruit pedicel length with any of the treatments.

TABLE 37

Effect of gibberellins, alone and in combination with other plant growth regulators applied in 1983 on fruit shape during the season and at harvest., number of seeds per fruit, length of pedicel and the number of flower buds in 1984 of Golden Delicious

Treatment	Length/diameter ratio		Number of seeds/ fruit	Length of pedicel mm	Number of flower buds/tree
	13 July	Harvest			
Control	1.073	0.993	3.8	32.0	717
GA_3	1.088	0.997	4.2	31.6	596
GA_{4+7}	1.133	1.023	3.7	32.0	592
GA_{4+7} + IAA	1.131	1.033	2.9	31.0	715
GA_{4+7} + NAA	1.122	1.033	2.4	33.4	748
GA_{4+7} + BA	1.150	1.044	2.5	31.8	699
SED	0.011	0.010	0.3	1.0	178

SED = standard error of difference (35 d.f.)

The number of flower buds produced by the trees in 1984 was not affected by any of the treatments. Results of the assessment of the quality of treated fruit after a period of air storage are given in Table 38. The fruit treated with GA_{4+7} + NAA was larger ($P<0.01$) than the control fruit, no other treatment effects being detected. All treatments resulted in fruit being less firm after storage, except for

that treated with GA_{4+7} + NAA, with GA_3 and GA_{4+7} being significantly ($P<0.05$) different from the control and the difference measured with GA_{4+7} + IAA and GA_{4+7} + BA being highly significant ($P<0.001$). Weight loss during storage was reduced ($P<0.001$) by the GA_{4+7}, GA_{4+7} + IAA and GA_{4+7} + BA treatments. No physiological disorders were apparent in any of the fruit after storage.

TABLE 38

Effect of gibberellins, alone and in combination with other plant growth regulators applied in 1983 on mean fruit weight, fruit firmness, weight loss and incidence of physiological disorders in fruit of Golden Delicious after a period of storage in air

Treatment	Mean fruit weight ex store g	Fruit firmness kg	Weight loss %	Physiological disorders %
Control	130.4	1.85	6.1	0
GA_3	133.6	1.78	5.9	0
GA_{4+7}	133.9	1.78	5.5	0
GA_{4+7} + IAA	134.5	1.74	5.6	0
GA_{4+7} + NAA	142.9	1.80	5.8	0
GA_{4+7} + BA	135.2	1.68	5.7	0
SED	4.4	0.03	0.1	-

SED = standard error of difference (35 d.f.)

1984

There was a higher incidence of russeting in this experiment compared to 1983 as shown by the low proportion of fruit in russet grade I (Table 39). All treatments reduced ($P<0.001$) the incidence of russeting as shown by the increase of 22-25% in the proportion of fruits in russet grades I + II. No significant differences between

TABLE 39

Effect of gibberellins GA_{4+7}, alone and in combination with other plant growth regulators applied in 1984 on fruit quality, number of fruit harvested, mean fruit weight, final yield and number of flower buds in 1985 of Golden Delicious

Treatment	Proportion of fruit in russet grade		Number of fruit harvested		Mean fruit weight	Final yield /tree	Number of flower buds/tree	
	I % Wt	I + II % Wt	/100 clusters	/tree	g	kg	spurs and terminals	auxillary
Control	1.2	55.2	195.3	792	117.5	92.2	2045	893
GA_{4+7}	3.2	78.6	176.4	664	120.4	79.1	1633	697
Enriched GA_{4+7}	4.6	83.8	164.8	673	126.8	84.6	1685	822
GA_{4+7} + NAA(1)	2.6	77.9	148.2	581	132.1	76.4	1823	888
GA_{4+7} + NAA(2)	5.4	79.9	132.5	538	145.8	77.9	1995	996
GA_{4+7} + BA	4.3	80.3	150.1	628	129.9	79.5	1580	745
SED	-	3.9	13.1	50	3.3	6.3	217	138

SED = standard error of difference (35 d.f.). - SED not valid.

NAA(1) - 1 mgl^{-1}, NAA(2) - 2.5 mgl^{-1}

the treatments were detected.

Some thinning activity was measured with all treatments. GA_{4+7} did not affect the number of fruit harvested/100 blossom clusters but reduced ($\underline{P}<0.05$) the number harvested/tree, while the enriched GA_{4+7} caused significant ($\underline{P}<0.05$) reductions in both parameters. Both GA_{4+7} + NAA treatments reduced the number harvested/100 blossom clusters and per tree ($\underline{P}<0.001$ except for GA_{4+7} + NAA(1) with number/100 clusters, $\underline{P}<0.001$), the GA_{4+7} + BA also resulting in reduced numbers ($\underline{P}<0.01$) of both parameters.

All treatments increased mean fruit weight recorded at harvest ($\underline{P}<0.01$ for enriched GA_{4+7}, $\underline{P}<0.001$ for others) except for GA_{4+7} (Table 39). The GA_{4+7} + NAA(2) produced significantly ($\underline{P}<0.01$) larger fruit than the GA_{4+7} + NAA(1) treatment and ($\underline{P}<0.001$) when compared to the other treatments. The final yield/tree was reduced ($\underline{P}<0.05$) by the GA_{4+7}, GA_{4+7} + NAA(1) and GA_{4+7} + NAA(2) treatments, no other significant treatment effect being recorded.

The number of flower buds produced on either two year or older wood or on one year old shoots in 1985 was unaffected by any treatment, except for a significant ($\underline{P}<0.05$) reduction in the former by the GA_{4+7} + BA treatment.

The thinning action of the various treatments resulted in a decrease in the proportion of fruits graded-out under 60mm in diameter and in the 60-65mm size category (Table 40). The proportion of fruit graded-out under 60mm in diameter was lower with the enriched GA_4, GA_{4+7} + NAA(1), GA_{4+7} + NAA(2) and GA_{4+7} + BA treatments compared to the control, but there was no effect of GA_{4+7} alone. All treatments

reduced the proportion of fruit graded-out in the 60-65mm size category, with the GA_{4+7} + NAA(2) treatment resulting in a decrease of over 70% compared to the control. The treatments also caused a corresponding increase in the proportion of fruits graded-out in the 70-75mm and over 75mm size categories.

TABLE 40

Effect of gibberellins GA_{4+7}, alone and in combination with other plant growth regulators applied in 1984 on the proportion of Golden Delicious fruits graded-out in different size categories

Treatment	Size grades mm.				
	% <60	% 60-65	% 65-70	% 70-75	% >75
Control	12.3	30.5	38.8	15.7	2.6
GA_{4+7}	12.9	24.5	39.0	19.2	4.4
Enriched GA_{4+7}	7.9	20.2	41.3	25.4	5.2
GA_{4+7} + NAA(1)	5.8	16.5	39.6	30.5	7.5
GA_{4+7} + NAA(2)	3.0	8.9	31.4	37.9	18.8
GA_{4+7} + BA	7.2	18.9	38.6	27.8	7.5
SED	-	-	2.3	2.8	-

SED = standard error of difference (35 d.f.). - SED not valid

NAA(1) - 1 mgl^{-1}, NAA(2) - 2.5 mgl^{-1}

4.4. DISCUSSION

Although the generally good skin finish of the fruit in 1983 made it difficult to detect any treatment effects, GA_4 and GA_{4+7} appear to be slightly more effective in the control of russet and cracking of Cox's Orange Pippin than GA_7 and GA_3, in agreement with the findings of Wertheim (1982) for Karmijn de Sonnaville and Golden Delicious. Other workers have found GA_3 to be less effective than GA_{4+7} (Eccher, 1978;

Taylor, 1978). The differences observed in this work and elsewhere between GA_4 and GA_7 were small, however, and this may explain the lack of any difference between the ordinary and GA_4 enriched GA_{4+7} formulations in 1984.

The varying degrees of russet control in response to the various gibberellins cannot be explained, but differences in uptake and/or subsequent metabolism in the plant tissues are possible factors, or it may be related to differences in tissue sensitivity. Wertheim (1982) mentions that GA_4 and GA_7, which tend to be more active in russet control than GA_3, are found naturally in apple fruit whereas GA_3 is not, but more recent work has established that all three are present in the seeds of young apple fruitlets (Hedden and Hoad, 1985).

The expectation that the addition of other plant growth regulators to GA_{4+7} would improve the control of russeting was not realised, at least not conclusively. Thus GA_{4+7} + NAA mixtures improved the skin finish of Golden Delicious to a greater extent than GA_{4+7} alone in 1983, but not in 1984 when the concentrations of NAA were reduced. The results obtained in 1983 corresponded with those mentioned for GA_{4+7} + 2,4,5-TP mixtures by Byers et al (1983), who found the mixture improved the skin finish of Golden Delicious compared to the components applied alone, although the results were limited to one trial. It is interesting to note that IAA was non-effective in comparison to NAA in this respect and this may be related to its relatively less stable structure, as it is known that IAA is rapidly decarboxylated in apple when applied in daylight (Grochowska, 1974). IAA has also been reported to be ineffective for russet control by Eccher (1975).

The results obtained with GA_{4+7} + BA correspond with those mentioned by Eccher (1983) and Steenkamp et al, (1984), the cytokinin component giving no apparent improvement of skin finish. Indeed, where cytokinins have been applied alone the incidence of russeting has generally been increased (Eccher, 1975; Taylor, 1975; McLaughlin and Greene, 1984). Eccher (1975) has associated the effects of cytokinins on russeting with disruption of the growth of the epidermis, resulting in an uneven cuticle layer, which makes the fruit more prone to the initiation of russet.

As in the previous chapter gibberellin treatment was shown to have a fruit thinning effect in some trials, the magnitude of this effect being increased dramatically where NAA was included. This is not surprising, however, since the commercial use of NAA as an apple fruit thinning agent is well established (Williams, 1979). Although mixtures of gibberellins and auxin have not been judged to be useful for improved russet control in this work, the improved thinning action of a combination of GA_{4+7} + NAAm on Golden Delicious has been shown to have practical applications for thinning this cultivar, while also improving the skin finish of the fruit (Wertheim, 1986a).

The various gibberellins clearly had different effects on fruit shape (Figure 2), although there was some inconsistency depending on the stage of fruit development. It is apparent that GA_3 has the least effect, in agreement with other work (Stembridge and Morell, 1972; Boffelli and Eccher, 1978), while the differences between the other gibberellins are small. Wertheim (1982) noted that fruitlets were more elongated early in the growing season, especially where treated with GA_4 or GA_{4+7} but no measurements were taken to support these

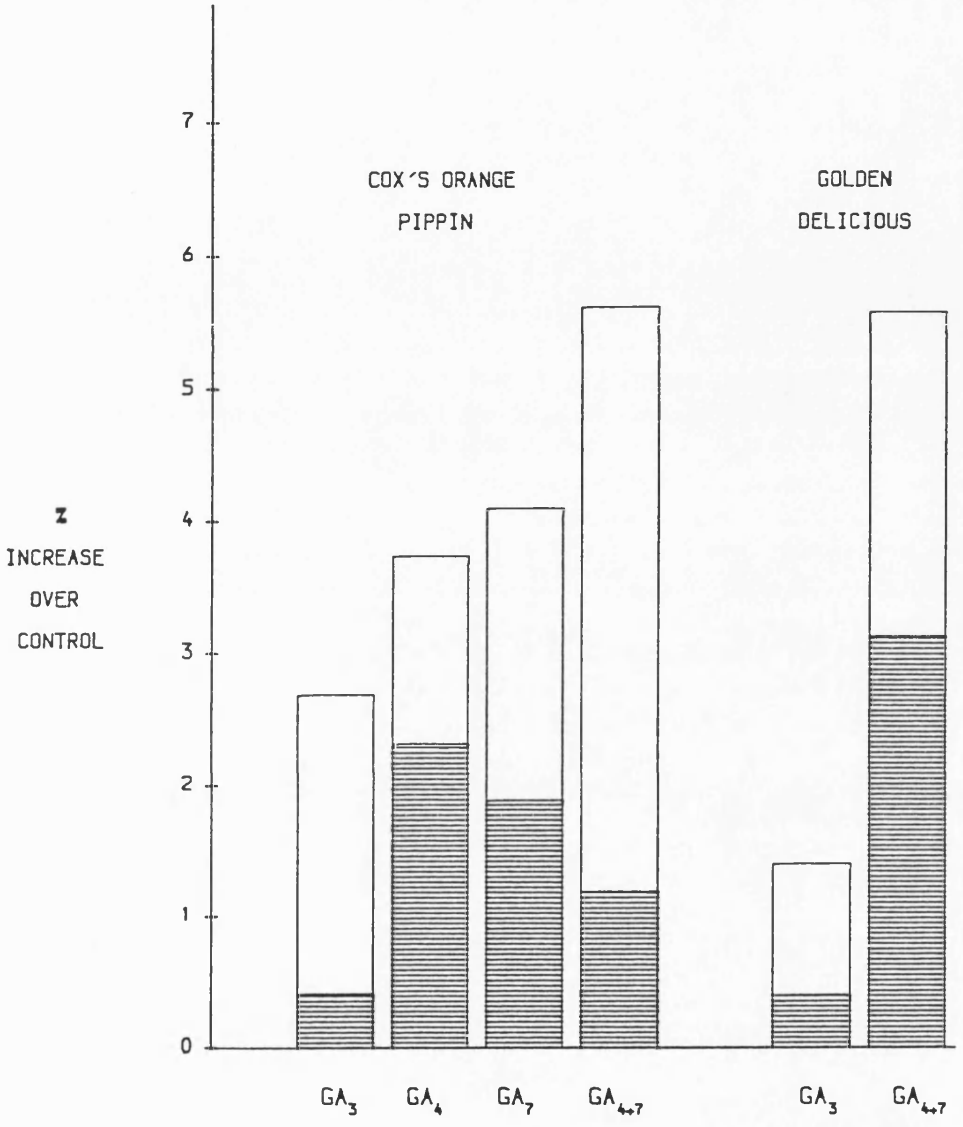


Fig. 2. Increase in the length/diameter ratio of fruit from trees of two cultivars sprayed with various gibberellins in 1983, measured in July ☐ and at harvest ▤

observations.

The combinations of plant growth regulators had a greater effect on the shape of Golden Delicious fruit, especially at harvest (Figure 3). The greatest response in both cultivars occured with the GA_{4+7} + BA combination, in agreement with the results of some work (Williams and Stahly, 1969; Stembridge and Morell, 1972), but in contrast to others (Greenhalgh et al, 1977; Eccher, 1983). The shape of apple fruit can be affected by a number of different factors (Dennis, 1986) and this may explain the varying response to applied plant growth regulators.

Althcugh no significant differences were recorded between the various gibberellins in their effects on return bloom, as has been generally demonstrated when low concentrations have been used (Wertheim, 1982), the results tend to support the differences reported after application of high concentrations. Thus GA_4 has little effect on flower bud numbers, whereas GA_7 has a significant inhibitory effect and GA_3 tends to be less inhibitory than GA_{4+7} (Tromp, 1982, 1987). Interestingly the GA_4 enriched GA_{4+7} formulation seems to be little different to GA_{4+7} in this respect and it is possible that the small proportion of GA_7 in the former is enough to inhibit blossoming.

The contrasting effects of the various gibberellins on flowering in apple have been attributed to their structural differences and corresponding differences in metabolism within plant tissues (Pharis and King, 1985). There are many gaps in our knowledge of the process of flower induction, however, and to establish the precise role of gibberellins in that process will require a sound knowledge of the

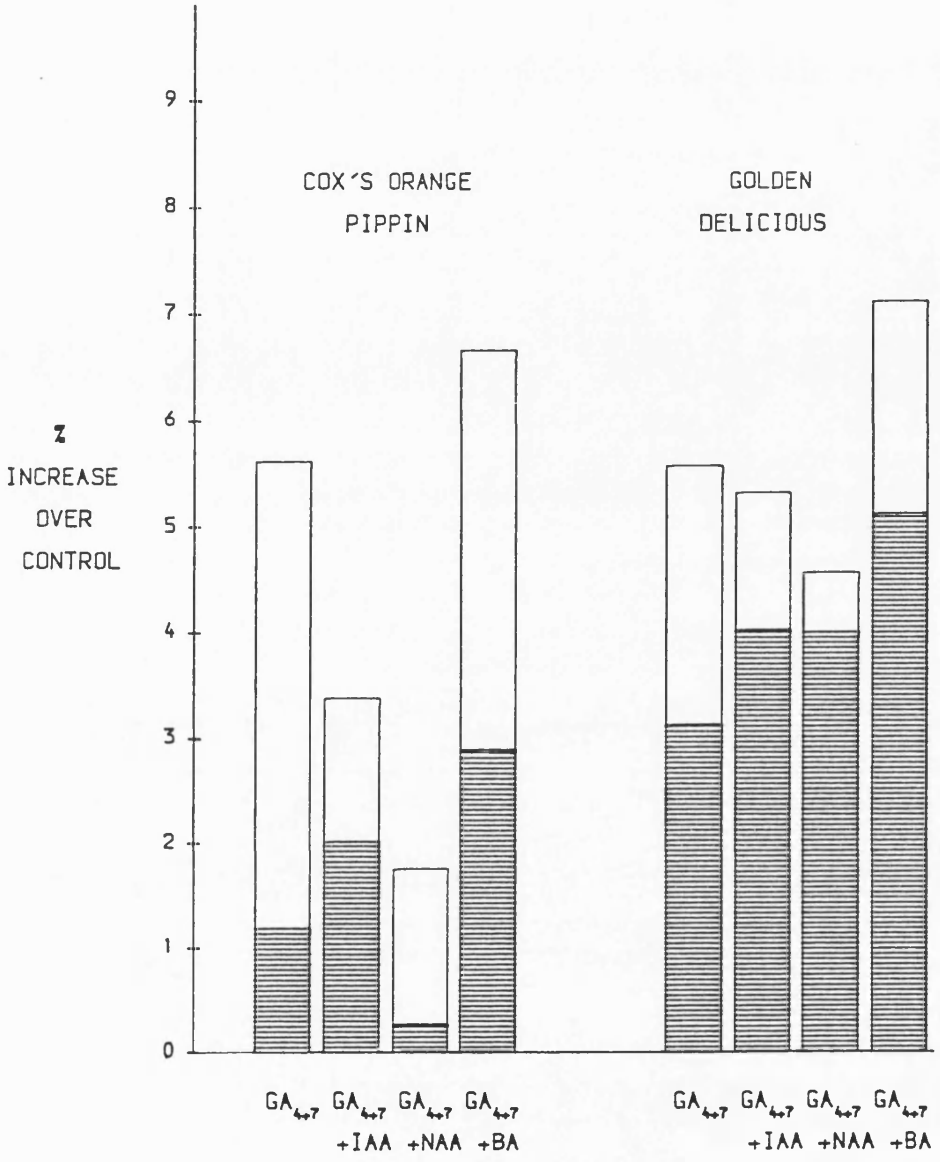


Fig. 3. Increase in the length/diameter ratio of fruit from trees of two cultivars sprayed with GA_{4+7} alone and in combination with other plant growth regulators in 1983, measured in July ☐ and at harvest ▤

hormonal content of tissues prior to and during the induction period, including measurements at the cellular level.

There was little difference in the effects of the various plant growth regulator combinations on return bloom, although it is known that cytokinins can increase flower bud numbers (Ramirez and Hoad, 1978; McLaughlin and Green, 1984). There is also evidence that where both GA_{4+7} and BA are applied, the BA component can mitigate the inhibitory effect of the GA_{4+7} (Eccher and Castelli, 1982; McLaughlin and Greene, 1984).

Although firmness was reduced in fruit of Golden Delicious treated with GA_{4+7} + BA after storage, there was no increase in the incidence of physiological disorders, in contrast to reports of marked increase in breakdown of stored fruit of other cultivars after a similar treatment (Looney, 1979; Greene et al, 1982). These effects were associated with low calcium levels within the fruit corresponding to a reduction in the number of seeds, the latter being recorded in this work but without any apparent detrimental effect on storage quality. The calcium levels of fruit were not determined in this study and so it is not possible to resolve this apparent discrepancy.

PART II - FUNDAMENTAL ASPECTS

CHAPTER 5

STUDIES ON THE EFFECTS OF GIBBERELLINS A_4 + A_7 AND/OR (2RS, 3RS) - PACLOBUTRAZOL ON THE DEVELOPMENT AND STRUCTURE OF THE SKIN TISSUES OF THE FRUIT OF APPLE CV GOLDEN DELICIOUS

5.1. INTRODUCTION

The gibberellins constitute a large group of diterpene acids and are known to be of widespread and probably universal occurrence in higher plants (Jones and MacMillan, 1984). In apple, a total of 24 gibberellins have now been identified in seeds and vegetative tissues using modern physico-chemical methods of analysis (Pharis and King, 1985), including GA_3, GA_4 and GA_7.

When applied to apple fruit skin gibberellins have been shown to affect not only the structure of the epidermal and cuticular layers (Eccher, 1975), but also to increase both cell division and cell expansion of the underlying cortical tissues (Bukovac and Nakagawa, 1968; Nakagawa et al, 1968). The effects on the skin tissues noted by Eccher (1975) included the appearance of a uniform layer of cuticle over a layer of more regular epidermal cells compared to the untreated fruit. However, no measurements of cell size, shape or thickness of cuticle were made and the effects were noted on fruit at harvest rather than early in the development of the fruit.

The plant growth retardant paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol] (coded PP333, commercial formulation Cultar; ICI Agrochemicals, Horticulture), is a potent and highly specific inhibitor of

gibberellins biosynthesis (Dalziel and Lawrence, 1984; Hedden and Graebe, 1985). Cultar is now commercially available for the chemical growth control of apple and pear trees (Lever, 1986). Apart from effects on shoot growth, applications of paclobutrazol have also been found to result in smaller fruit with a reduced length/diameter ratio, but subsequent foliar applications of GA_3 reversed these effects on Golden Delicious apple (Currey and Williams, 1983). There is also evidence that applications of paclobutrazol, especially if applied soon after petal-fall, can increase the incidence of russeting in fruit of Golden Delicious (Borsboom, 1983).

This chapter studies the effects of GA_{4+7} applications on the structure and development of the cuticular, epidermal and hypodermal layers of the apple fruit and relate these effects to the reduction in the incidence of russet and cracking. Because of the unusual specificity and potency of the effects of paclobutrazol on gibberellin biosynthesis, the chemical was used as a tool in this study.

5.2. MATERIALS AND METHODS

Ten year old Golden Delicious trees on M9 rootstocks at a spacing of 4.1 m x 2.5 m were treated in 1984 with (1) 25 mgl^{-1} GA_{4+7} on 25 May,4 June and 14 June; paclobutrazol at 1000 mgl^{-1} on (2) 25 May or (3) 1 June. Other trees received treatments (1) + (2) or (1) + (3), while control trees were left unsprayed. Single treatments were applied as overall sprays to whole trees, with two trees per treatment. Combination treatments were applied to labelled branches, the GA_{4+7} sprays being superimposed on trees that had received overall sprays of paclobutrazol. All sprays were applied with a hand-held sprayer to drip-point.

During the season, samples of ten fruit from each treatment were selected at random, five fruit from each tree. Collections were made on 13, 27 June, 13, 27 July and 10 October (harvest). At harvest the remaining fruit from each treatment was picked into boxes and a sample of 50 fruit (25 fruit per tree) was selected at random from each treatment. These were then assessed for russet; fruits were divided into three regions ie the stalk-end, the cheek and the eye-end and each region was assessed separately for russet using a four point scale:

grade 1 - no russet

grade 2 - <1/8 of area with russet

grade 3 - 1/8 - 1/4 of area with russet

grade 4 - >1/8 of area with russet

For each of the five treatments, the number of fruit in each of the four russet grades was recorded for each area of the fruit surface. This gave a 5 x 4 contingency table for each area and χ^2 tests were then used to assess whether the distribution of fruit across the russet grades was the same for each treatment.

A russeting index was then calculated by multiplying the number of fruit in grade 1 by 1, that in grade 2 by 3, that in grade 3 by 5 and that in grade 4 by 7, after which the figures were added. A single figure was thus produced which expressed the incidence of russeting per treatment, a lower figure corresponding with a lower incidence of russeting.

Length/diameter ratios of the fruit were also determined.

Sample Preparation

The material for histological examination was prepared as follows. Samples were either fixed on the day of collection or were stored in a refrigerator until this could be done.

Fruits were cut to a suitable size, usually one half or one quarter of the fruit being used. Samples were fixed for at least 24 hours in 4% formalin under vacuum infiltration and were stored in this solution until further preparation. Tissues were then dehydrated using a cellulose/ethanol/propanol/tertiary butyl alcohol series and embedded in 'Paramat' paraffin wax. Sections 8 µm thick were cut using a rotary microtome, the wax ribbons being floated on 4% formalin on slides previously smeared with Haupt's adhesive, warmed gently until the wax had fully expanded and then left to dry.

Sections were then de-waxed with xylene, hydrated and dehydrated with the usual series of alcohol and water mixtures and then stained using a solution of 0.5% chlorazal black in 70% ethanol, the period of staining varying from 45 minutes to 2 hours depending on the sample. Sections were then cleaned using 70%, 100% ethanol and xylene and were then mounted in Xam.

Measurements of the periclinal and anticlinal dimensions of ten cells from each fruit were made using a Reichert projection microscope, the data being analysed by analysis of variance. Photomicrographs were taken on Kodak Panatonic X film using a Wild M20 microscope.

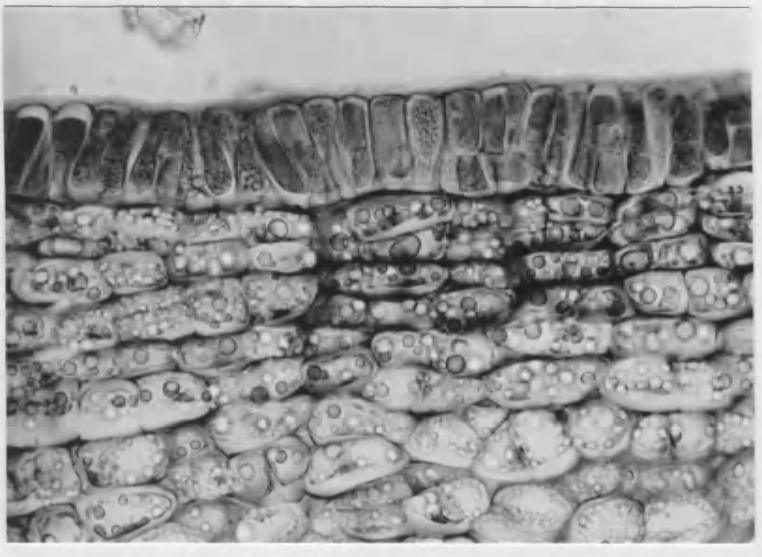
Plate 4 - Transverse sections of skin tissues from Golden Delicious fruit, untreated, sprayed with GA_{4+7} or paclobutrazol. Sampled on 13 June 1984. GA_{4+7} applied on 25 May and 4 June; paclobutrazol applied on 23 May.

50µm

GA_{4+7}

Control

Paclobutrazol



5.3. RESULTS

Plate 4 illustrates the differences apparent between the epidermal and hypodermal cells of fruit from the control, GA_{4+7} and paclobutrazol treatments on 13 June 1984.

Treatment with GA_{4+7} and/or paclobutrazol significantly influenced the size and shape of the epidermal cells (Table 41). GA_{4+7} caused a significant ($P<0.001$) increase in the periclinal dimensions of the cells, whereas paclobutrazol had the reverse effect ($P<0.001$) and the two treatments combined produced no overall effect. No treatment effect was detected on the anticlinal dimensions of the cells but the ratio of the periclinal/anticlinal dimensions was increased ($P<0.001$) by GA_{4+7}, decreased ($P<0.001$) by paclobutrazol and was unaffected where the the two treatments were combined. The size of the cells was increased significantly ($P<0.05$) by GA_{4+7}, reduced ($P<0.01$) by paclobutrazol and was unaffected where both were applied.

In samples taken on 13 June 1984 only paclobutrazol treatment affected the size and shape of the hypodermal cells (Table 42). The treatment increased ($P<0.05$) the periclinal dimensions, reduced ($P<0.01$) the anticlinal dimensions and increased ($P<0.01$) the periclinal/anticlinal ratio when compared to the control. The periclinal dimension was significantly ($P<0.01$) greater, the anticlinal dimensions reduced ($P<0.001$) and the periclinal/anticlinal ratio greater ($P<0.001$) when compared to GA_{4+7}.

TABLE 41

Effect of GA_{4+7} and/or paclobutrazol applied in 1984 on the dimensions and shape of epidermal cells of Golden Delicious fruit sampled on 13 June

Treatment	Cell dimensions µm		Periclinal/ anticlinal ratio	Cell size μm^2
	Periclinal	Anticlinal		
Control	10.3	26.2	0.40	270.9
Paclobutrazol(*)	8.8	26.2	0.33	234.8
GA_{4+7}	11.5	25.7	0.45	296.4
Paclobutrazol + GA_{4+7}	10.4	26.7	0.39	277.1
SED	0.3	0.7	0.01	11.2

SED = standard error of difference (16 d.f.)

(*) applied 23 May 1984

TABLE 42

Effect of GA_{4+7} and/or paclobutrazol applied in 1984 on the dimensions and shape of hypodermal cells of Golden Delicious fruit sampled on 13 June

Treatment	Cell dimensions µm		Periclinal/ anticlinal ratio
	Periclinal	Anticlinal	
Control	21.5	13.6	1.64
Paclobutrazol(*)	23.2	12.3	1.96
GA_{4+7}	20.9	14.2	1.53
Paclobutrazol + GA_{4+7}	20.6	13.1	1.62
SED	0.7	0.4	0.09

SED = standard error of difference (16 d.f.)

(*) applied 23 May 1984

In fruit sampled on 27 June 1984 the periclinal dimension of the hypodermal cells was increased ($P<0.05$) and ($P<0.001$) by paclobutrazol compared to the control and GA_{4+7} respectively (Table 43). Anticlinal dimensions of the cells were increased ($P<0.001$) by GA_{4+7} compared to the control and paclobutrazol. GA_{4+7} decreased ($P<0.01$), whereas paclobutrazol increased ($P<0.01$) the periclinal/anticlinal ratio, with the value being similar to the control where the two treatments were combined.

TABLE 43

Effect of GA_{4+7} and/or paclobutrazol applied in 1984 on the dimensions and shape of hypodermal cells of Golden Delicious fruit sampled on 27 June

Treatment	Cell dimensions µm		Periclinal/ anticlinal ratio
	Periclinal	Anticlinal	
Control	24.6	14.5	1.75
Paclobutrazol(*)	26.4	13.6	2.03
GA_{4+7}	23.7	16.6	1.45
Paclobutrazol + GA_{4+7}	25.5	15.5	1.69
SED	0.6	0.5	0.08

SED = standard error of difference (16 d.f.)

(*) applied 23 May 1984

Plate 5 illustrates the differences apparent between the incidence of russeting of fruit at harvest from the control, paclobutrazol and paclobutrazol + GA_{4+7} treatments.

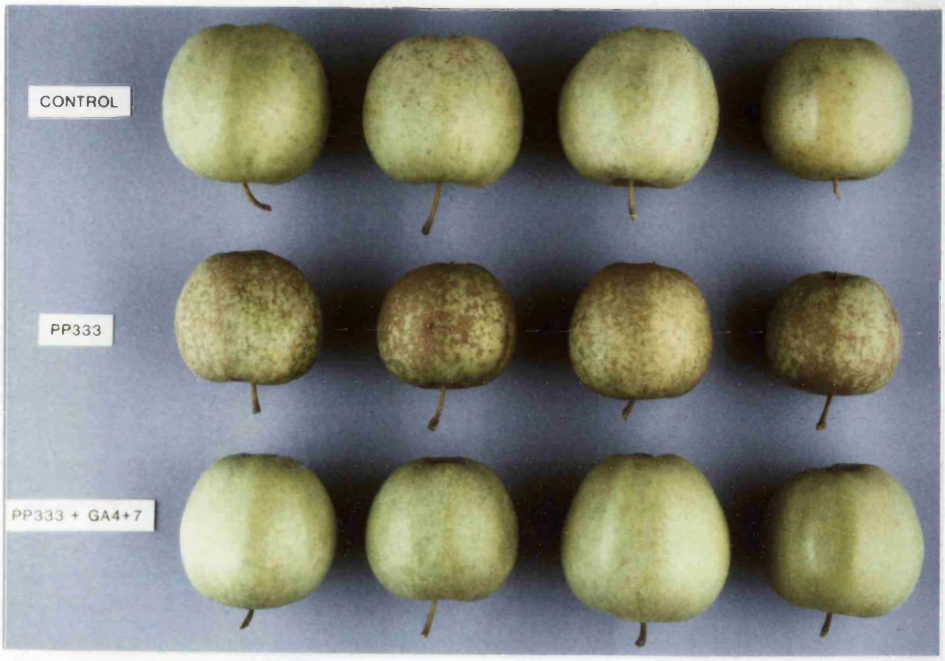


Plate 5 - Golden Delicious fruit, untreated (top), sprayed with paclobutrazol (middle) and paclobutrazol + GA_{4+7} (bottom). Paclobutrazol applied 23 May; GA_{4+7} applied 25 May, 4 June and 14 June

The effects of GA_{4+7} and/or paclobutrazol on russeting, fruit size and shape are recorded in Table 44. The incidence of russeting was significantly ($P<0.001$) affected by the treatments for all three areas of the fruit surface. It is clear that paclobutrazol increased the incidence of russeting, while the addition of GA_{4+7} reduced it for all three areas of the fruit surface, as shown by the increasing and decreasing russet scores respectively. The results also show that paclobutrazol applied on 23 May 1984 results in more russeting than where applied on 1 June 1984.

Both paclobutrazol treatments caused a significant ($P<0.01$) decrease in the length of the fruit, when compared to both control

fruit and fruit treated with GA_{4+7}. There was no treatment effect on fruit diameter but the length/diameter ratio was reduced ($P<0.05$) by both paclobutrazol treatments compared to the control and GA_{4+7} combined treatments.

TABLE 44

Effect of paclobutrazol and GA_{4+7} applied in 1984 on russeting, fruit size and shape of Golden Delicious

Treatment	Russet index			Fruit length mm	Fruit diameter mm	Length/ diameter ratio
	Calyx end	Cheek	Stalk end			
Control	192	143	130	59.2	60.2	0.983
Paclobutrazol (a)	234	276	218	53.0	57.0	0.932
Paclobutrazol (b)	208	172	172	53.7	57.6	0.932
Paclobutrazol (a) + GA_{4+7}	100	94	118	59.0	60.2	0.981
Paclobutrazol (b) + GA_{4+7}	92	104	112	58.4	59.0	0.992
SED				1.0	1.5	0.013
X^2	163.7	174.5	63.5			

SED = standard error of difference (5 d.f.)

Values of X^2 (12 d.f.) calculated for tables giving the number of fruit in each russet grade for each treatment

(a) applied 23 May 1984, (b) applied 1 June 1984

5.4. DISCUSSION

Preliminary studies in 1983 showed that GA_{4+7} applied in the immediate post-bloom period tended to increase the size of both epidermal and hypodermal cells and reduce the fluctuations in cuticle thickness as measured by a reduction in the number of deep cuticular flanges extending between the epidermal cells (Taylor et al, 1985). These observations made on young fruitlets soon after GA_{4+7} application

tend to support the findings of Eccher (1975), who observed similar effects in the fruit of Golden Delicious at harvest after GA_{4+7} applications in the blossom and post-bloom period. Increases in cell size of fruit tissues after gibberellin treatments have been reported in apple (Bukovac and Nakagawa, 1968; Nakagawa et al, 1968) and grape (Sachs and Weaver, 1968), although these reports refer to effects on the cells of the fruit flesh rather than the skin tissues.

Although these preliminary findings were supported by the results obtained in 1984, it is relevant to look at the direction of this increased growth of the cells, rather than the magnitude of the effect per se. Thus it is apparent, both from the measurements and the photomicrographs, that the treatments had dramatic effects on the shape of the epidermal cells, with GA_{4+7} and paclobutrazol having the opposite effect compared to the control. It is interesting to note that GA_{4+7} apparently enhanced the development of the fruit while paclobutrazol retarded it, if the shape of the epidermal cells is used as a measure of development. The cells are columnar in shape at petal-fall but their dimensions tend to equalise as the fruit develops (Bell, 1937a; Meyer, 1944).

The effects of GA_{4+7} and paclobutrazol on the hypodermal cells was equally dramatic, with again the two treatments having the opposite effect. Shape of hypodermal cells has been correlated with the incidence of russet and cracking in Cox's Orange Pippin, with increased incidence of the disorders where the periclinal/anticlinal ratio of the cells was high (Skene, 1962), and the results reported here correspond with these observations. Skene mentions the 'stretched' appearance of the hypodermal cells, which is apparent in the photomicrograph of the paclobutrazol treated fruit, and suggested that the cells were under

stress causing straining in the fruit tissues which became apparent as russet. This response, it was supposed, was caused by the production of a compound or compounds, possibly hormonal, which initiated the development of a periderm. This would form under the intact cuticle, a pattern of events differing markedly from normal russet initiation in which it is the rupture of the cuticle and the subsequent exposure of the underlying cells that leads to the formation of a periderm (Faust and Shear, 1972a). The weight of evidence in the literature, however, tends to support the view that in partially russeted cultivars, russet forms by the latter mechanism.

The effects of the treatments on cell shape are reflected in changes in the morphology of the fruit as a whole. Since changes in fruit shape have been recorded with associated changes in skin finish of the fruit after growth regulator treatments, as in these studies, it has been suggested that the two may be interrelated (Wertheim, 1982). A possible explanation for such a relationship comes from studies into the effect of fruit shape on the magnitude and direction of surface growth forces that may develop in the fruit during growth (Considine and Brown, 1981). According to this work changes in fruit shape will alter the direction and magnitude of the stresses and the resultant strains that develop in the skin tissues during development. Since strain is greatest at the outermost edge of a growing cell or organ (Probine and Preston, 1961), an understanding of the shape of a fruit and how this changes during development or after a particular treatment is applied, should enable predictions to be made of the amount and direction of cracking that will appear in the skin tissues (Considine

and Brown, 1981).

A survey of the literature on russeting and cracking of apple fruit indicates that no attempt has been made to measure stress in the skin tissues of developing fruit in the period immediately after flowering. Skene (1980a) measured stress in developing fruit of Cox's Orange Pippin and found no measurable stress until the fruits were approximately 15mm in diameter, but measurements were not taken of fruit below approximately 10mm diameter. Hence there is no information relating to the earliest stages of fruit development, which is known to be the critical period for russet initiation and when control measures, such as GA_{4+7}, must be applied to be fully effective. Thus it is difficult to discuss the possible relationship between fruit shape and russeting, although it should be noted in this context that GA_{4+7} applications had little measurable effect on fruit shape of Discovery apples, yet significantly improved the skin finish (see Chapter 2).

The effects of the growth regulator treatments on cell size and shape in the skin tissues of apple fruit are probably related to effects on cell expansion. Gibberellins are generally thought to promote growth in higher plants by increasing cell expansion or cell division or both, but the primary effect must be on the former (Jones, 1982), due in most cases to increased cell-wall extensibility. Effects of GA_{4+7} and paclobutrazol on the cell wall extensibility of apple fruit skin tissues may, in turn, be related to the observed effects on russeting of the fruit and this will be discussed in the next chapter.

Application of paclobutrazol in the period immediately after flowering clearly increases the incidence of apple fruit russeting,

corresponding with other work (Borsboom, 1983). This is not surprising, however, since it is known that paclobutrazol is a potent inhibitor of gibberellin biosynthesis, and subsequent applications of GA_{4+7} completely reversed the deleterious effects on skin finish, as well as the effects on fruit shape. Applications of GA_3 and GA_{4+7} + BA have also been shown to overcome the effects of paclobutrazol on apple fruit shape (Curry and Williams, 1983). Increased risk of russeting is a major reason for the recommendation to avoid applying paclobutrazol for growth control purposes in apple orchards within three weeks of petal-fall (Lever, 1986).

CHAPTER 6:

PRELIMINARY STUDIES ON THE EFFECTS OF GIBBERELLINS A_4 + A_7 AND/OR (2RS,3RS)-PACLOBUTRAZOL ON THE RHEOLOGICAL PROPERTIES OF THE SKIN TISSUES OF FRUIT OF APPLE CVS GOLDEN DELICIOUS AND COX'S ORANGE PIPPIN

6.1. INTRODUCTION

Gibberellins can promote shoot growth in plants, an effect which, it has been argued, can be related to their effect on cell expansion, cell division or both (Jones, 1973). It is clear, however, that gibberellin enhanced tissue elongation cannot be explained in terms of cell division (Green, 1976). Tissues increase in volume because cells increase in volume: thus without cell expansion there can be no elongation and therefore the primary effect of gibberellins must be on cell expansion. Cell division simply limits the potential for growth and final volume (Jones, 1982).

The growth of plant cells can be described in terms of five parameters and the steady state growth rate Vs can be described by the equation:

$$Vs = \frac{L\Phi}{L+\Phi} (\sigma\Delta\pi - Y)$$

where L = hydraulic conductance of the cell; Φ = cell wall extensibility; σ = solute reflection coefficient; $\Delta\pi$ = difference in osmotic potential across the cell membrane and Y = yield threshold, which is defined as the maximum tugor pressure for growth (Cosgrove, 1981). Experimental evidence suggests that gibberellin enhanced growth is related either to changes in $\Delta\pi$ or Φ.Thus Cleland *et al* (1968) found that GA_3 stimulated the growth of cucumber hypocotyl tissue by

affecting H_2O uptake, whereas Adams et al (1975) with Avena internodes and Kawamura et al (1976) and Stuart and Jones (1977) working with lettuce hypocotyls, have shown that GA_3 increased cell wall extensibility. Jones (1982) has suggested that only in those tissues which show a dramatic response to gibberellin, does the hormone enhance wall extensibility.

Many techniques have been used to measure cell wall extensibility (Cleland, 1981) but the most widely used is the Instron technique, pioneered by Olson et al (1965). This gives a measurement of both the elastic and plastic extensibilities of the tissues and it is the latter which is usually equated with Φ (Cleland, 1971, 1981). The tissue under test is subjected to two successive extensions at a constant rate of strain and the stress along the cell walls is recorded as a function of extension. The first extension involves both a reversible and an irreversible component, while the second extension is entirely reversible. The slope of each curve (% extension/unit stress) is measured at a particular stress; the slope of the second curve gives the elastic extensibility (EEx), while the difference in slopes between the two extensions is the plastic extensibility (PEx) (Cleland, 1967). In experiments using Avena coleoptiles and soybean hypocotyl walls, Cleland (1984) found that the PEx determined by the Instron technique appears to be a measure of the average Φ which existed in the hour or so preceding the time the tissue was harvested.

Although Instron machines which are purpose built to measure extensibility are commercially available, they suffer from the drawbacks of being expensive and are usually designed to measure considerably tougher materials than plant tissues (Van Volkenburgh et

al, 1983). The purpose of the work described in this chapter was to develop a simple extensometer that would measure the extensibility of apple fruit skin tissues. This was then used to measure the effects of GA_{4+7} and/or paclobutrazol on the extensibility of the skin tissues during the period immediately after flowering, to gain an understanding of the possible mode of action of these plant growth regulators in relation to their effects on russeting and cracking.

6.2. MATERIALS AND METHODS

Extensometer Design

The instrument (Figure 4) was designed to produce load-extension relationships comparable to stress-strain curves. Samples were held between clamps (A) constructed from spring loaded paper clips (10 x 20 mm). The lower clamp was fixed and samples were extended by raising the upper clamp. Movement of this clamp was controlled by means of a water filled syringe (B), the clamp being connected to the syringe piston (C) via a movable shaft (D) and a horizontal brass cantilever bar (E). Water was introduced into and withdrawn from the syringe at a controlled rate via a peristaltic pump (Polystaltic Pump; Buchler Instruments, Fort Lee, New Jersey, USA) with low pulsing characteristics. The displacement of the clamp, (and therefore the extension of the sample), was determined by means of a linear displacement transducer (G), (LVDT type D5/100 : RDP Electronics Limited, Wolverhampton, UK), to which the movable shaft was also attached. The load that developed when the sample is extended was measured by two 8 mm copper/nickel alloy strain gauges (H) (RS Components Limited, London, UK), cemented by means of quick-set epoxy resin adhesive to the upper and lower faces of the cantilever bar. The

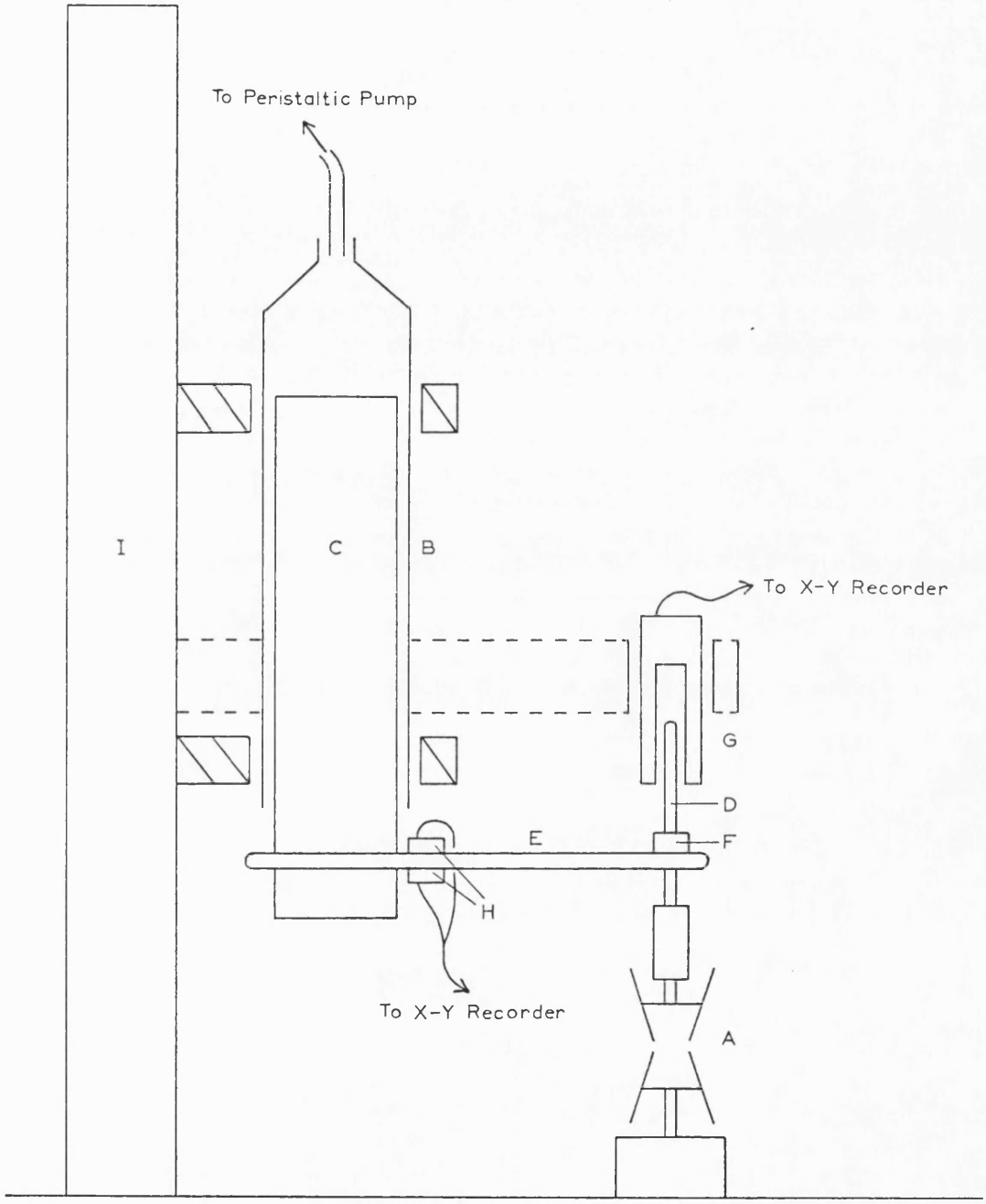


Fig. 4. Diagram of the extensometer. Labelled parts are (A) clamps, (B) syringe, (C) syringe piston, (D) movable shaft, (E) brass cantilever bar, (F) locking nut, (G) position transducer or LVDT, (H) strain gauges and (I) stand.

strain gauges, as a pair, automatically compensated for any thermal expansion strains that developed in the cantilever bar.

The equipment was mounted on a stable, rigid stand (I), the syringe/upper clamp assembly and the LVDT being attached with separate clamping systems. The stand allowed positional adjustment of the extensometer relative to the lower fixed clamp.

The output from the strain gauges was amplified by a DC amplifier plus power supply (Strain Gauge Amplifier No. 308-815 + DC power supply : RSC Limited), the output from the LVDT being amplified by means of a transducer amplifier (RDP Electronics Limited). Both amplified outputs were displayed on an XY chart recorder (Bryans, Model 29000 A4); the Y axis represented the output from the strain gauges, the X axis that from the LVDT, load-extension relationships being obtained.

Growth Regulator Treatments

Eleven year old Cox's Orange Pippin trees on M9 rootstocks were sprayed with 10 mgl^{-1} GA_{4+7} on 22 May (80% petal-fall), 29 May, 5 June and 13 June 1985. Eleven year old Golden Delicious trees on M9 rootstocks were sprayed as follows: (1) 10 mgl^{-1} GA_{4+7}, (2) 100 mgl^{-1} GA_{4+7}, (3) 500 mgl^{-1} paclobutrazol and (4) treatments (1) + (3). GA_{4+7} treatments were applied on 23 May (50% petal-fall), 29 May, 5 June and 13 June 1985, paclobutrazol being sprayed on 21 May 1985. Treatments were replicated five times with both cultivars, control trees being unsprayed. Six to ten fruit per replicate were taken at random from all sides of the trees on the 1st and 6th June and placed as soon as possible into a freezer (-18^{o}C) until required.

Previous work with Avena coleoptiles treated with indole-3-acetic acid (IAA) showed that although the freeze-thawing process may affect the actual extensibility of the tissues, the relative effects of the treatments applied remains constant irrespective of the treatment used to prepare the tissues (Cleland, 1984). This was assumed to be the case with the tissues involved in these experiments.

Procedures

1. Sample Preparation

The frozen samples were packed in ice and transported to the University of Bath for extensibility measurements. After thawing at room temperature the stalk and calyx end of the fruit were removed with a razor blade and samples of skin tissue taken from the remaining part of the fruit both in a longitudinal direction (between stalk and calyx ends) and transversely (around the equator of the fruit). In the case of Golden Delicious the skin tissues separated relatively easily from the flesh such that strips of skin were removed simply by pulling with fine tweezers. This was not the case with Cox's Orange Pippin; the samples of skin tissue were cut from the fruit with a razor blade and as much as possible of the adhering flesh removed. In both cases the separated strips of skin tissue were stored in distilled water until required. Prior to the extensibility tests the strips were cut to a uniform size using fixed parallel razor blades; transverse sections were trimmed to 2 x 15 mm and longitudinal sections to 1.5 x 9 mm.

In practice, it was only possible to prepare samples from fruit of both cultivars picked on the 6th June, as those picked on the 1st June were found to be too small for this purpose. The average diameter of fruit picked on the 6th June was approximately 10mm.

2. Extensibility Measurements

Plate 6 shows the upper and lower clamp assemblies, the upper clamp being to the left. In order to grip the specimens with the minimum damage to the tissues, liners consisting of two pieces of thin, smooth aluminium sheeting (13 x 10 mm) joined together with tape to form a hinged unit are used. The liner in the lower clamp was permanently attached to the clamp with double-sided sellotape.

To load the specimen into the upper clamp, one end of the specimen was inserted into the open liner and gripped by holding the two sections of the liner together with a spring clip. The liner with the specimen was then inserted into the upper clamp, which was then closed to hold the liner and specimen, the spring clip being removed. The free end of the specimen was then inserted (but not fixed) into the open clamp, the distance between the clamps being then adjusted to a specified gap by moving the upper clamp; the gap between the clamp was measured, with plastic feeler gauges, to be 7.5 and 4 mm for the transverse and longitudinal sections respectively. The lower clamp was then closed.

The sample was extended by moving the upper clamp at a rate of 1.25 mm min^{-1} up to a maximum loading; 20 and 10 g for transverse and longitudinal specimens respectively. After maximum loading was reached, the clamps were returned to their original positions. The procedure was then repeated to produce a typical load-extension curve (Cleland, 1967). To prevent dehydration of the tissue during this procedure, a drop of distilled water was placed on to the specimen prior to extension.

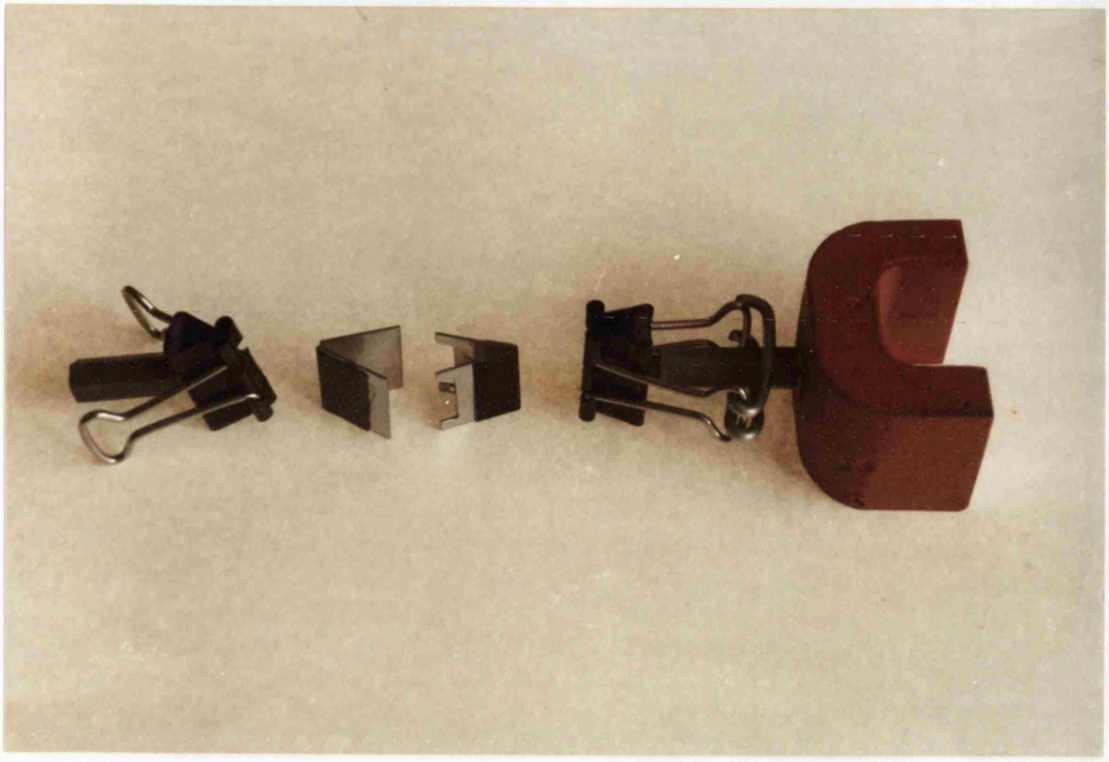


Plate 6 - Upper and lower clamp assemblies, upper clamp to the left.

The displacement rate was calibrated with the aid of a travelling microscope which measured the displacement of the upper clamp over a given time interval. The loading was calibrated by hanging weights to the upper clamp and noting the reading on the X-Y recorder. Calibration was carried out at the beginning and end of every session.

The extensibility of the specimens was determined as described for the Instron technique (Cleland, 1967; Van Volkenburgh et al, 1983). Extensibility is calculated from the slope of the load-extension curve

eg for a specimen loaded to 20 g:

$$\text{extensibility} = \left(\frac{lf-li}{li} \times 100\right) \Big/ 2 = \underset{10g \text{ load}}{\% \text{ change in length per}}$$

where lf and li are the final and initial lengths of the sample.

The first extension is a measurement of the total (elastic + plastic) extensibility, the second extension being a measurement of elastic extensibility only. Plastic extensibility is therefore calculated from the difference between the two measurements.

Usually there was a finite amount of slack in the sample prior to extension, which meant that although the clamps were a set distance apart, the initial length of the specimen was actually slightly greater. The actual initial length of each specimen was obtained by extending the slope of the load-extension curve back to the X-axis, the intercept providing the true value.

At least ten samples per treatment were tested, each from different fruit, the set of figures being averaged and standard errors calculated. The significance of the difference between two sample means was calculated using a t test.

Variation in extensibility may be attributable to differences in sample thickness (Van Volkenburgh et al, 1983). Calculation of cell wall surface area is difficult with higher plant tissues, but may be approximated by a conversion from specific tissue weight determined from deproteinized tissues (Cleland, 1967). No attempt was made to do this in the present studies, but the samples were dried in an oven after measurements of extensibility had been carried out and weighed

when dry. No differences in the weight of the samples were apparent between the treatments (data not presented).

6.3. RESULTS

There was found to be insufficient material within the samples of fruit to enable both transverse and longitudinal sections to be measured, hence only results obtained with the former are presented.

Golden Delicious

Applications of 10 mgl^{-1} GA_{4+7} increased ($P<0.05$) the EEx of the skin tissues compared to the untreated controls by approximately 10%, but this was not true for fruit treated with 100 mgl^{-1} GA_{4+7} (Table 45). The EEx of skin tissues of fruit treated with paclobutrazol was approximately 12% lower than the control value ($P<0.001$), while that of skin tissues of fruit treated with paclobutrazol and GA_{4+7} was similar to the control.

TABLE 45

Effect of GA_{4+7} and/or paclobutrazol applied in 1985 on the extensibility of the skin tissues of Golden Delicious fruit, assessed in transverse samples from fruit picked on 6th June

Treatment	Number of replicates	Extensibility (% extension/10 g load)	
		Elastic	Plastic
Control	15	2.47 ±0.05	1.19 ±0.09
GA_{4+7} (10 mgl^{-1})	15	2.74 ±0.09	1.49 ±0.06
GA_{4+7} (100 mgl^{-1})	10	2.48 ±0.06	1.45 ±0.06
paclobutrazol (500 mgl^{-1})	10	2.16 ±0.04	0.96 ±0.05
paclobutrazol (500 mgl^{-1}) + GA_{4+7} (10 mgl^{-1})	10	2.52 ±0.07	1.25 ±0.08

Treatment with 10 mgl^{-1} GA_{4+7} resulted in approximately 25% increase in the value of PEx of the skin tissues compared to the control ($P<0.05$) but no significant effect was found where 100 mgl^{-1} GA_{4+7} was used. Although the PEx of skin tissues from fruit treated with paclobutrazol was lower than the control value, the difference was not significant. This was significantly lower, however, when compared to the GA_{4+7} ($P<0.001$) and the paclobutrazol + GA_{4+7} effects ($P<0.01$), showing aproximately 35% reduction when compared to the 10 mgl^{-1} GA_{4+7} treated skin tissues. The PEx of skin tissues of fruit treated with paclobutrazol + GA_{4+7} was similar to that obtained from the untreated controls.

Cox's Orange Pippin

The results from the experiment with this cultivar are given in Table 46. Both the EEX and PEx values obtained were considerably lower than those of Golden Delicious but no significant treatment effects were found.

TABLE 46

Effect of GA_{4+7} applied in 1985 on the extensibility of the skin tissues of Cox's Orange Pippin fruit, assessed in transverse samples from fruit picked on 6th June

Treatment	Number of replicates	Extensibility (% extension/10 g load)	
		Elastic	Plastic
Control	16	1.76 ±0.05	0.69 ±0.03
GA_{4+7} (10 mgl^{-1})	16	1.71 ±0.05	0.80 ±0.05

6.4. DISCUSSION

The work described here demonstrates the successful construction of an instrument capable of measuring the cell-wall extensibility of skin tissues from young apple fruitlets and the use of this instrument to measure the effects of plant growth regulators on the rheological properties of the skin from Golden Delicious and Cox's Orange Pippin fruitlets. The GA_{4+7} was shown to have increased both EEx and PEx of the skin tissues of Golden Delicious fruit, while paclobutrazol had the reverse effect. Increases in cell-wall extensibility in various plant tissues after treatment with gibberellin have been recorded (Adams et al 1975; Kawamura et al, 1976; Stuart and Jones, 1977). In other tissues, however, no effect was recorded and increased growth of the tissues was attributed to changes in the osmotic potential of the tissues (Cleland et al, 1968). The mechanisms involved whereby gibberellins can alter the cell-wall extensibility of plant tissues remain to be elucidated, although a number of possibilities have been proposed (see Jones and MacMillan, 1984).

Although it was not found to be possible with the technique developed, to measure the effects of plant growth regulators on the rheological properties of skin tissues of apple fruit at the very early stages of development, the measurements obtained suggest that effects on cell-wall extensibility may be responsible for the observed effects on russeting and cracking of apple fruit. An increase in the cell-wall extensibility of the epidermal and hypodermal cells could be expected to enable these tissues to accommodate to a greater degree any stress that may arise in the skin during fruit development . This in turn would reduce the likelihood of mechanical failure of the skin tissues

caused by the strains induced in the tissues by stress. Also, any disruption to the epidermal cells caused by such strain could be expected to affect in turn the development of the cuticle layer, since it is presumed that the components of the cuticle and its subsequent deposition are as a result of epidermal cell metabolism and secretion (Miller, 1982). Hence increased cell-wall extensibility would mitigate against the factors responsible for russet initiation in apple cultivars where partial russet was induced by environmental conditions (Faust and Shear, 1972a) and seems likely to be a fundamental factor responsible for the improved skin finish after GA_{4+7} treatments have been applied.

It seems reasonable to suppose that as paclobutrazol inhibits gibberellin biosynthesis, then the endogenous gibberellin levels in treated apples are likely to be deficient, resulting in an increased risk of russet initiation due to decreased cell-wall extensibility, as indeed is suggested by the results reported here. As has been shown previously, GA_{4+7} and paclobutrazol have opposite effects on russeting of apple fruit. These effects correspond to changes in skin tissue extensibility, including treatments in which both growth regulators are applied together, where GA_{4+7} counteracts the effects of paclobutrazol both on russeting and on extensibility. This can be seen as further supportive evidence for the role of cell-wall extensibility in the relationship between gibberellins and russeting.

The work reported here is the first recorded attempt to examine the rheological properties of the skin tissues from young developing apple fruits, previous work being limited to the examination of mature

fruits (Clevenger and Hamann, 1968). Other studies have measured the rheological properties of the skin from various fruits, in an attempt to relate this to the susceptibility of the skin to develop cracking. These studies have included measurements of cherry (Levine et al, 1959), grape (Hankinson et al, 1977) and tomato (Batal et al, 1970; Voisey et al, 1970; Hankinson and Rao, 1979). However, in these studies, the tensile strength of the skin has usually being determined rather than its extensibility, the skin tissues being tested to failure.

CHAPTER 7:

STUDIES ON THE UPTAKE AND TRANSLOCATION OF APPLIED GIBBERELLINS IN APPLE CVS GREENSLEEVES, COX'S ORANGE PIPPIN AND GOLDEN DELICIOUS

7.1. INTRODUCTION

Plant growth regulators are widely used in fruit production, and with various objectives, but a frequent limitation to their general use is a high degree of inconsistency in their performance. The basis for the inconsistent response is not clear but has been related to spray application and the subsequent uptake and translocation of the growth regulator. The application of an exact, uniform dose to the target is a prerequisite for uniform response, yet the process of spray application is complex (Bukovac, 1985).

Various aspects of the uptake and translocation of exogenous gibberellins in fruit crops have been studied using [^{14}C] labelled GA_3, including work on cherry (Cristoferi and Filiti, 1983; Knight and Webster, 1986), pear and plum (Knight and Webster, 1986), orange (Goldschmidt and Galili, 1981, Greenberg et al, 1984) and grapefruit (Ferguson et al, 1986). Studies in apple have been limited to those of Hoad (1978), who investigated the movement of GA_3 after injection into seeds within developing apple fruitlets, while Goldwin (1984) examined the uptake and movement of exogenous GA_3 in the flowers of Cox's Orange Pippin.

The purpose of the work described in this chapter was to examine the uptake and translocation of exogenous gibberellins in apple fruits and shoots in relation to the control of russeting and cracking. The studies included investigations into the uptake of applied gibberellins

by apple fruit when applied to the fruit skin and the subsequent movement within the fruit tissues, together with the uptake and translocation within a fruit cluster when applied to cluster leaves. The majority of this work involved the use of [^{14}C]-GA_3 or [^{3}H]-GA_4, but also included studies with localised applications of GA_{4+7}. [^{14}C]-GA_3 was used in the early experiments as this was the only labelled GA commercially available at the time, [^{3}H]-GA_4 not being obtained until 1985.

7.2. MATERIALS AND METHODS

EXPERIMENTS WITH [^{14}C] AND [^{3}H] LABELLED GIBBERELLINS

In the experiments conducted in 1984 [1,7,12,18-^{14}C] GA_3, (specific activity 555 MBq m mole; radiochemical purity, 98%: Amersham International, Amersham, UK), was used throughout. The original ethyl acetate solution of [^{14}C]-GA_3 was dried down by passing nitrogen over the required volume contained in a glass vial, then re-dissolved in a small volume of propylene glycol. This was then made up to the required dilution for each experiment with an aqueous solution of 50 mgl^{-1} GA_3 plus 0.1% Tween 20 as a wetter.

Experiments were conducted under field conditions using the apple cultivar Greensleeves (Golden Delicious x James Grieve), the trees being nine years old on MM106 rootstocks at a spacing of 4.5 m x 3.0 m. Treatments were replicated five times in randomised block designs, with trees acting as blocks. The treatments were applied to uniform, undamaged clusters via a micro-pipette (Gilson-'Pipetman' 0-20µl model).

When experiments were harvested, treated leaves and/or fruit were

washed with three 1 ml aliquots of distilled methanol and wiped with glass-wool soaked in distilled methanol. Plant tissues were freeze dried and the radioactivity recovered by combusting in a Harvey biological oxidiser (model OX200); the recovered [^{14}C] was determined by liquid scintillation counting (Beckman model LS7800). Radioactivity in the washings and glass-wool was also determined by scintillation counting after the addition of 10 ml of scintillation fluid (Beckman Ready-Solv EP). An external standard quench correction procedure was used, counting efficiency varying between 75-85%. Data was corrected for efficiency and expressed as disintergrations per minute (DPM). The figures obtained were averaged and standard errors calculated.

In the experiments conducted in 1985 [1,2(n)-^{3}H] GA_4, (specific activity 1.11 TBq m mole; radiochemical purity, 92.6%: a gift from Dr P Hedden, Long Ashton Research Station, Long Ashton, Bristol, UK), was used throughout. The [^{3}H]-GA_4 was supplied as an ethyl acetate solution and prepared using the same procedure described earlier for [^{14}C]-GA_3, except that it was made up to the required dilution for each experiment with an aqueous solution of GA_{4+7} plus 0.1% Tween 20.

Experiments were conducted under field conditions using the cultivars Cox's Orange Pippin and Golden Delicious, each cultivar receiving identical treatments. The Cox's Orange Pippin trees were five years old on M9 rootstocks at a spacing of 4.0 m x 3.0 m; Golden Delicious trees were eleven years old on M9 rootstock at a spacing of 4.1 m x 2.5 m. Treatments were replicated five times in randomised block designs, with trees as blocks, and were applied as described previously for the 1984 experiments. The plant tissues were treated after harvest as described previously for the 1984 experiments, the

scintillation counting efficiency varying between 15-50%.

Uptake-Time Course Experiment - 1984

On 9 June two 10µl aliquots of the GA_3 solution containing 2.03 k Bq of [^{14}C] GA_3 were applied to the surface of the receptacle of one fruit on each of 25 clusters. Fruit was harvested after 1, 3, 6, 24 and 48 hours and treated as described previously. The average fruit diameter at the time of the experiment was 15 mm.

Effect of Concentration of Carrier Solution on Uptake Experiment - 1985

Solutions of 2.5, 5 and 10 mgl^{-1} GA_{4+7} were prepared with the [^{3}H]-GA_4; a 10µl droplet of each solution containing 2.77 kBq of [^{3}H]-GA_4 was applied to the surface of the receptacle of one fruit in each of thirty clusters. Fruit was harvested 4 and 24 hours after treatment. Treatments were applied on 28 May when the average fruit diameter was 7.3 mm and 6.2 mm for Cox's Orange Pippin and Golden Delicious respectively.

Movement Within a Fruit. Experiment 1 - 1984

On 19 June one 15µl droplet of GA_3 solution containing 3.7 kBq [^{14}C]-GA_3 was applied to the receptacle surface of one fruit on each of fifteen clusters. The treated area was marked with waterproof ink, the fruit being harvested 4, 24 and 72 hours after treatment. Treated fruit were divided into five longitudinal sections (A-E) with a razor blade, section A containing the area where the [^{14}C]-GA_3 was applied and E the section on the opposite side of the fruit. Section E was always cut first, working towards section A and the razor blade was rinsed with distilled methanol after each incision. The average fruit diameter in the experiment was 23.5 mm.

Experiment 2 - 1985

On 8 June a 10µl droplet of 100 mgl^{-1} GA_{4+7} solution containing 7.4 kBq of [^{3}H]-GA_4 was applied to the surface of the receptacle of one fruit in each of ten clusters. The treated area was marked with waterproof ink, the fruit being harvested 48 and 120 hours after treatment. Treated fruit were divided into three longitudinal sections (A-C) with a razor blade, section A containing the area where the [^{3}H]-GA_4 was applied and C the section on the opposite side of the fruit. Section C was always cut first, working towards section A and the razor blade was rinsed with distilled methanol after each incision. The average fruit diameters in the experiment were 15.5 mm and 13.9 mm for Cox's Orange Pippin and Golden Delicious respectively.

Translocation Within a Cluster. Experiment 1 - 1984

On 10 June five 10µl aliquots of GA_3 solution containing a total of 11.1 kBq [^{14}C]-GA_3 were applied to the abaxial surface of one mature leaf of each of five clusters. The treated clusters were harvested after 76 hours and divided into separate components of leaves, fruit, bourse and peduncle prior to determination of radioactivity in the tissues.

Experiment 2 - 1985

On 25 May five 10µl aliquots of a 100 mgl^{-1} GA_{4+7} solution containing 7.4 kBq of [^{3}H]-GA_4, were applied to the abaxial surface of one mature leaf of each of ten clusters. Treated clusters were harvested after 48 and 120 hours and divided into leaves, fruit, bourse and peduncle. The fruits were further divided by cutting transversly with a razor blade and removing the central area containing the seeds with a scalpel. Dissecting instruments were rinsed with distilled

methanol after each dissection.

EXPERIMENTS WITH LOCALISED APPLICATIONS OF GA_{4+7}

The experiment involved the use of paclobutrazol as a russet-inducing agent which formed the control treatment. Ten year old Golden Delicious trees on M9 rootstocks at a spacing of 4.1 m x 2.5 m were used. One hundred and sixty blossom clusters on twenty trees were labelled immediately after full-bloom on 19 May 1984. The 'king' flower and all except two lateral flowers were then removed, the remaining flowers being hand pollinated with James Grieve pollen.

Trees were divided into two groups of ten; paclobutrazol (1000 mgl^{-1}) was applied to eighty clusters on trees in one group on 23 May (50% petal-fall), and to eighty clusters on trees in the second group on 1 June. Three GA_{4+7} treatments were then superimposed on the paclobutrazol treatment in both groups of trees. A screen was constructed from a rectangular, plastic plate approximately 30 x 15 cm, which was cut to the centre where a small hole large enough to accommodate the peduncle had been drilled. The screen was placed between the fruitlets and the cluster leaves to allow application of 25 mgl^{-1} GA_{4+7} solution to either the fruit or leaves independantly. Both fruit and leaves were treated with the screen removed, each GA_{4+7} treatment being applied to twenty clusters in each group of trees, leaving twenty clusters which had received paclobutrazol alone. The GA_{4+7} treatments were applied on 25 May, 4 and 14 June, using a hand-held sprayer to drip point. Trees acted as blocks with treatments being replicated twenty times, two clusters per tree receiving each treatment.

Fruit was harvested on 9 October and assessed for russet. Each fruit was assessed in three regions ie the stalk-end, the cheek and the eye-end, with each region being assessed for russeting using a four point scale:

grade 1 - no russet.

grade 2 - <1/8 of area with russet.

grade 3 - 1/8 to 1/4 of area with russet.

grade 4 - >1/8 area with russet.

The figures obtained were added and the total divided by the total number of fruit in each treatment to give an average grade figure for each area of the fruit. In the experiment the number of replicates differed from treatment to treatment, due to the large number of fruit that dropped during the season. However, a single approximate SED was calculated for each variate.

Length/diameter ratios of the fruit were also determined.

7.3. RESULTS

EXPERIMENTS WITH [^{14}C] AND [^{3}H] LABELLED GIBBERELLINS

Uptake-Time Course Experiment - 1984

The uptake of [^{14}C] was initially rapid, with a significant proportion being detected in the fruit after 3 hours (Table 47). Very little uptake occurred in the next 3 hours, whereas a considerable amount of [^{14}C] was taken up between 6 and 24 hours after [^{14}C]-GA_3 application. No further uptake occurred after 24 hours. No [^{14}C] was detected in the untreated fruit on the cluster up to 48 hours after the [^{14}C]-GA_3 was applied.

Effect of Concentration of Carrier Solution on Uptake Experiment - 1985

There was no effect of carrier solution concentration on the

uptake of [^{3}H] in either Cox's Orange Pippin or Golden Delicious fruit (Table 48). The amount of [^{3}H] taken up doubled in the case of Cox's Orange Pippin and nearly trebled in Golden Delicious between 4 and 24 hours after the application of the [^{3}H]-GA_4.

TABLE 47

Uptake of [^{14}C] with time by apple fruits cv Greensleeves after treatment with [^{14}C]-GA_3 in 1984

Time after application hours	Radioactivity recovered	
	dpm	% of that applied
1	17100 ±4345	13.9
3	47416 ±4442	38.7
6	50202 ±4416	41.0
24	79080 ±7998	64.5
48	69654 ±2274	56.8

After 4 hours the amounts of [^{3}H] taken up by Cox's Orange Pippin was approximately three times that taken up by Golden Delicious, but after 24 hours the difference was reduced to being only twice as much.

Movements Within a Fruit. Experiment 1 - 1984.

The uptake of [^{14}C] was found to increase with time, over 60% of the total [^{14}C] applied being detected within the fruit after 48 hours (Table 49). In general the amount of [^{14}C] detected in the sections of the fruit showed a negative relationship with the distance from the point of application of the [^{14}C]-GA_3. Over 90% of the [^{14}C] taken up over the 72 hours of the experiment remained in section A, with little evidence of significant movement of [^{14}C] away from the point of application. No [^{14}C] was detected in section E, furthest from the

point of application and only a trace amount was detected in section D after 72 hours.

TABLE 48

Uptake of [^{3}H] in fruit of apple cvs Cox's Orange Pippin and Golden Delicious after application of [^{3}H]-GA_4 in various concentrations of carrier GA_{4+7} solution in 1985

GA_{4+7} conc mgl^{-1}	Radioactivity recovered - dpm			
	Cox's Orange Pippin		Golden Delicious	
	hours after application		hours after application	
	4	24	4	24
2.5	42383 ±3775 (23.7)	81637 ±7982 (45.8)	15670 ±1634 (8.8)	42433 ±2116 (23.8)
5.0	44407 ±4061 (28.1)	79001 ±5846 (50.0)	14877 ±1051 (9.4)	39249 ±4147 (24.8)
10.0	39811 ±2134 (24.0)	74778 ±8000 (45.1)	14454 ±1375 (8.7)	39499 ±2816 (23.8)

Values in brackets represent the % of that applied.

TABLE 49

Distribution of [^{14}C] within fruit of apple cv Greensleeves at various times after treatment with [^{14}C]-GA_3 in 1984

Section of fruit	Radioactivity recovered - dpm		
	hours after application		
	4	24	48
A	5888 ±1120	90421 ±7416	144063 ±11334
B	255 ±124	5058 ±1961	11533 ±1900
C	279 ±87	432 ±84	890 ±95
D	-	-	*
E	-	-	-

* <0.1% activity recovered. - no activity

Experiment 2 - 1985

In contrast to the previous experiment the amount of [^{3}H] detected in the fruit of both cultivars did not increase over the period of the experiment (Table 50); a maximum uptake of approximately 28% of the total [^{3}H] applied occurred with Golden Delicious and approximately 13% in Cox's Orange Pippin. Distribution of [^{3}H] within the fruit of both cultivars showed a negative relationship with the distance from the point of application of the [^{3}H]-GA_4, with only a small proportion of the [^{3}H] taken up being found in section C. There was some evidence of movement of [^{3}H] away from the point of application between 48 and 120 hours after treatment, with a slight increase in the amount of [^{3}H] detected in section C in both cultivars.

TABLE 50

Distribution of [^{3}H] within fruit of apple cvs Cox's Orange Pippin and Golden Delicious at various times after treatment with [^{3}H]-GA_4 in 1985

Section of fruit	Radioactivity recovered - dpm							
	Cox's Orange Pippin				Golden Delicious			
	hours after application				hours after application			
	48		120		48		120	
A	40722	±9762	37041	±4634	62557	±8687	58573	±14191
B	13163	±2696	17694	±5960	54813	±14232	31831	±8848
C	687	±231	1802	±469	4555	±744	6655	±2505

Translocation Within a Cluster Experiment 1 - 1984

After the 72 hours of the experiment approximately 70% of the [^{14}C] applied was found within the tissues of the first cluster, with around 68% being detected in the treated leaf (Table 51). A small but significant amount of [^{14}C] was detected in the fruit, with smaller amounts in the peduncle at the end of the experiment. There was some evidence of acropetal movement of trace amounts of [^{14}C] to the leaves above the treated leaf, but no basipetal movement to lower leaves was detected.

TABLE 51

Distribution of [^{14}C] within a cluster of apple cv Greensleeves 72 hours after treatment of a leaf with [^{14}C]-GA_3 in 1984

	Radioactivity recovered	
	dpm	% of that recovered
treated leaf	444412 ±26498	97.6
+ leaves	*	*
- leaves	-	-
peduncle	1445 ±262	0.3
fruit	9757 ±1489	2.1

* <0.1% activity recovered. - no activity

+ leaves - those above the treated leaf

- leaves - those below the treated leaf

Experiment 2 - 1985

The amounts of [^{3}H] taken up by both cultivars was the same 48 hours after treatment with approximately 30% of that applied being detected in the different components of the cluster, but 120 hours after treatment the total amount of [^{3}H] present in Cox's Orange Pippin had decreased by approximately 50%, whereas the amount in Golden Delicious had increased by around 25% (Table 52).

In Cox's Orange Pippin the major proportion of the [^{3}H] taken up remained in the treated leaf over the course of the experiment, with small but significant amounts being detected in the bourse shoot and peduncle, both after 48 and 120 hours. Traces of [^{3}H] were detected in the fruit at both times of sampling but there was no evidence of either acropetal or basipetal movement of [^{3}H] to other leaves.

TABLE 52

Distribution of [^{3}H] within a cluster of apple cvs Cox's Orange Pippin and Golden Delicious at various times after treatment of a leaf with [^{3}H]-GA_4 in 1985

	Radioactivity recovered - dpm			
	Cox's Orange Pippin		Golden Delicious	
	hours after application		hours after application	
	48	120	48	120
treated leaf	148195 ±30368 (96.5)	95408 ±18028 (97.6)	111560 ±2622 (91.7)	174354 ±21680 (89.3)
+ leaves	-	-	*	1622 ±334 (0.6)
- leaves	-	-	*	688 ±192 (0.3)
bourse shoot	1566 ±578 (1.4)	722 ±336 (0.7)	*	609 ±166 (0.3)
peduncle	2782 ±292 (2.1)	1678 ±422 (1.7)	6475 ±1210 (5.3)	9485 ±2438 (4.8)
fruit	*	*	3472 ±809 (2.9)	9226 ±2064 (4.7)

* <0.1% activity recovered. - no activity

+ leaves - those above the treated leaf

- leaves - those below the treated leaf

Similarly, the major proportion of the [^{3}H] taken up was detected in the treated leaf of Golden Delicious, although the proportion declined slightly over the course of the experiment. Significant amounts of [^{3}H] were detected in the peduncle and fruit 48 hours after treatment

and the amounts had increased by approximately 50 and 100% respectively when measured 120 hours after treatment. A trace amount of [^{3}H] was detected in the bourse shoot 48 hours after treatment and this increased to a small but significant level after 120 hours. Evidence of both acropetal and basipetal movement of [^{3}H] to other leaves was found, especially 120 hours after treatment but the amounts detected were small.

EXPERIMENTS WITH LOCALISED APPLICATIONS OF GA_{4+7}

Results from the trees where paclobutrazol was applied on 23 May 1984 are given in Table 53. The incidence of russeting was reduced by all the treatments when compared to fruits that had received paclobutrazol alone, this being true for all the areas of the fruit surface except for the calyx-end when GA_{4+7} had been applied to the leaves only. GA_{4+7} applied to the fruit + leaves resulted in significantly less russeting compared to where GA_{4+7} was applied to the leaves only, this being true for the cheek ($P<0.001$, SED 0.2) and the calyx-end ($P<0.001$, SED 0.3). Overall, GA_{4+7} applied to both the fruit and leaves resulted in the lowest russet scores for all areas of the fruit surface.

The length/diameter ratio of the fruit was increased ($P<0.001$) by the treatments where GA_{4+7} was applied to the fruit only and to fruit and leaves, compared to that treatment with paclobutrazol alone or where GA_{4+7} was applied to the leaves only.

TABLE 53

Effect of GA_{4+7} and/or paclobutrazol applied in 1984 on russeting and fruit shape of Golden Delicious fruit

Treatment	Russet score			Length/ diameter ratio
	Stalk-end	Cheek	Calyx-end	
paclobutrazol	3.1	3.6	3.0	0.862
GA_{4+7} - fruit	2.3	1.5	1.5	0.923
GA_{4+7} - leaves	2.3	2.3	2.7	0.879
GA_{4+7} - fruit + leaves	2.0	1.1	1.2	0.947
SED'S	0.2	0.2	0.3	0.019
GA_{4+7} treatments vs paclobutrazol only	0.2 0.3	0.2 0.2	0.3 0.3	0.019 0.020

SED = standard error of difference (46 d.f.)

SED'S relate to GA_{4+7} treatments vs paclobutrazol alone

The results from the trees where paclobutrazol was applied on 1 June 1984 are given in Table 54. Russeting was reduced in all areas of the fruit where GA_{4+7} was applied to the fruit and leaves, the russet scores being lower at the stalk-end the the cheek ($P<0.01$), as well as at the calyx-end ($P<0.001$). Where GA_{4+7} was applied to the fruit only this resulted in significantly less russet on the cheek ($P<0.01$) and at the calyx-end ($P<0.001$) but had no effect on that at the stalk-end compared to the paclobutrazol treated fruit. There was no effect on russeting where GA_{4+7} was applied to the leaves only except for a significant ($P<0.05$) reduction at the calyx-end.

TABLE 54

Effect of GA_{4+7} and/or paclobutrazol applied in 1984 on russeting and fruit shape of Golden Delicious fruit

Treatment	Russet score			Length/ diameter ratio
	Stalk-end	Cheek	Calyx-end	
paclobutrazol	2.8	2.4	3.1	0.919
GA_{4+7} - fruit	2.4	1.3	1.4	0.976
GA_{4+7} - leaves	2.6	2.2	2.5	0.927
GA_{4+7} - fruit + leaves	1.9	1.1	1.0	0.982
SED'S	0.3	0.3	0.3	0.020
GA_{4+7} treatments vs paclobutrazol only	0.3	0.3	0.3	0.020
	0.3	0.4	0.3	0.020

SED = standard error of difference (33 d.f.)

SED'S relate to GA_{4+7} treatments vs paclobutrazol alone

Significantly ($P<0.05$, SED 0.3) less russeting occurred at the stalk-end where GA_{4+7} was applied to the fruit and leaves compared to where it was applied to the leaves only. Treatment with GA_{4+7} of the fruit and the fruit and leaves resulted in significantly less russeting on the cheek ($P<0.01$, SED 0.3; $P<0.01$, SED 0.4) and at the calyx-end ($P<0.001$, SED 0.3; $P<0.001$, SED 0.3) when compared to that treated with GA_{4+7} on the leaves only. As with the previous experiment, GA_{4+7} applied to the fruit and leaves resulted in the lowest russet scores for all areas of the fruit surface.

Application of GA_{4+7} to the fruit only and to fruit and leaves together increased ($P<0.05$) the length/diameter ratio of the fruit compared to that treated with paclobutrazol, GA_{4+7} applied to the leaves only having no effect.

7.4. DISCUSSION

The basis for the inconsistent response frequently encountered when plant growth regulators are applied to tree fruits is not clear, but it is believed that absorption of the chemical into the plant tissues is a key contributing factor (Bukovac et al, 1986). The present work demonstrated that there is an initially rapid uptake of applied gibberellin into apple fruit tissues followed by a reduced rate of uptake, a pattern that corresponds with results from other plant species, both with gibberellins (McComb, 1964; Ferguson et al, 1986) and other growth regulators (Bukovac, 1973). This pattern of uptake has been generally associated with the drying of the spray droplet, with rapid uptake when the chemical is present in solution, a reduced rate as the droplet dries probably related to increased osmotic pressure as the chemical concentration increases, and little or no uptake after the residue has formed. Continued penetration from the residue may take place depending on the relative humidity (Luckwill and Lloyd-Jones, 1962), as indicated in the present studies where further uptake occurred overnight, when relative humidity, possibly leading to the formation of dew, could be expected.

In contrast with results obtained with other plant growth regulators (Bukovac, 1973), uptake of gibberellin was not enhanced with increasing concentration of the solution. This result is of interest as it may help to explain the apparent lack of response with increasing GA_{4+7} concentrations in regard to the control of russet and cracking (see Chapter 2). That Cox's Orange Pippin absorbed more gibberellin than Golden Delicious could be due to differences in the surface characteristics of the fruits of the two cultivars, possibly related to

the nature of the cuticular waxes. Also the Golden Delicious fruit were at an earlier stage of development compared to those of Cox's Orange Pippin and consequently the density of the hairs on the fruit surface would be higher in the former (Bell, 1937a). The surface of the Golden Delicious fruit would therefore be more difficult to wet, thus affecting uptake.

The results obtained suggest that gibberellins move within apple fruit tissues by diffusion, as has been shown with other fruit (Ferguson et al, 1986). This would explain the variation in the results obtained in 1984 and 1985, whereby there was apparently greater mobility in fruit in 1985 compared to 1984. The fruit of Greensleeves used in 1984 were much larger than the fruit of the two cultivars in 1985, and hence the gibberellin would have to move through a larger volume of tissue when applied to the Greensleeves fruit.

Uptake of gibberellins by leaf tissues was considerable and it is probable that this would be greater through the abaxial compared to the adaxial surface of the leaf (Greenburgh et al, 1984). The stomata present in the abaxial surface will contribute to greater penetration, although it is the guard and accessory cells, rather than the stomatal pores, which serve as the main route of entry (Bukovac, 1973).

The clear evidence of vascular transport of gibberellins from leaves to fruits correspond with results obtained in both apple (Hoad, 1978), and other plant species (Zweig et al, 1961; McComb, 1964; Cristoferi and Filiti, 1983; Ferguson et al, 1986). The apparent lack of transport to the fruit of Cox's Orange Pippin compared to the other cultivars is difficult to explain. A possible explanation is that the

comparative strengths of the fruit and leaves acting as 'sinks' for the attraction of carbohydrates and amino acids could have varied between the cultivars at the time of the experiments, the gibberellins moving towards the stronger sink. This has been suggested as the mechanism responsible to explain the movement of GA_3 in fruits and shoots of sweet cherry, with greater movement towards fruit or shoots depending on the stage of growth during the season (Cristoferi and Filiti, 1983).

Further evidence for vascular transport of gibberellins from leaves to fruits comes from the work with localised applications of GA_{4+7}, although as with labelled gibberellins the amount of movement was small. Thus there was only a slight indication of an improvement in the skin finish of fruit where GA_{4+7} was applied only to the leaves. This corresponds with the results obtained with localised applications of GA_{4+7} + BA to increase the length/diameter ratio of apple fruit (Greene, 1984), where small but significant increases were recorded when treatments were applied to the leaves but significantly greater response was obtained when they were applied to the fruit. In the present studies length/diameter ratios of the fruit were increased only where GA_{4+7} was applied to the fruit and not when applied only to the leaves.

Although no attempt was made in this work to identify either $[^{14}C]$-GA_3 or $[^{3}H]$-GA_4 in the labelled material recovered, other work suggests that applied gibberellins are rapidly metabolised after entry into plant tissues (eg Davies and Rappaport, 1975). $[^{3}H]$-GA_4 is rapidly metabolised after injection into the bourse shoots of apple (Looney et al, 1978) and by analogy with the results obtained with structuraly similar gibberellins, is likely to be more rapidly metabolised than GA_7 (Pharis and King, 1985).

CHAPTER 8:

GENERAL DISCUSSION

The quality of apple fruit is now of primary importance to growers if they are to maintain a profitable business in the present-day marketing situation, where Class I fruit attract a large premium and lower quality fruit can be difficult, if not impossible, to sell. One component of apple fruit quality is skin finish, and russeting and cracking of the fruit skin is a major cause of downgrading in susceptible cultivars such as Cox's Orange Pippin, leading to considerable financial loss to the grower. This research has demonstrated the potential of gibberellins $A_4 + A_7$ for significantly improving the skin finish of susceptible apple cultivars and has indicated the mode of action of such treatments in relation to the reduction of russeting and cracking.

Spray applications of low concentrations (<5 mgl^{-1}) of GA_{4+7} were found to give significant improvements in the skin finish of fruit of Cox's Orange Pippin, Golden Delicious and Discovery, with little or no apparent benefit from increasing concentration. Other effects of GA_{4+7} treatments were recorded including reductions in the number of fruit, and increases in their length/diameter ratio, and inhibition of flower initiation (Chapter 2). These effects, where they occurred, were more apparent where high concentrations of GA_{4+7} had been applied and it is evident that control of russeting and cracking with low concentrations of GA_{4+7} is possible without the occurrence of deleterious side-effects to any degree (Wertheim, 1982). The effectiveness of low concentrations of GA_{4+7} is probably because the fruit skin is exposed directly to the spray treatment, requiring only penetration and minimal

transport of the gibberellins to the site of action. Thus the potential for dilution of the gibberellins, for example by their metabolism and/or preferential transport to other parts of the plant, will be minimal. This situation is analogous to that in which GA_3 is used successfully to delay the senescence and treat various disorders of the rind of citrus fruit (Coggins, 1982).

The timing and number of applications used was found to be crucial if the optimum effect of GA_{4+7} was to be achieved, in agreement with other studies (Taylor, 1978; Eccher and Boffelli, 1981). At least four spray applications were found to be necessary and the first spray should be applied within the flowering period (Chapter 3). Although considerable work was carried out to investigate the relative importance of timing and number of applications, the results obtained were limited by the natural good skin finish of all the fruit in these trials. Further experiments will be needed to establish the optimum combination of number of sprays, interval between sprays and timing for UK conditions, since other work has shown that efficacy can be increased by the use of the correct combination of these factors (Dijke and Kester, 1983). An important consideration in this context, however, will be the practical feasibility of implementing any GA_{4+7} spray programme in commercial apple production.

The addition of other plant growth regulators to GA_{4+7} did not improve the control of russeting and cracking (Chapter 4), although there was some evidence of an anti-russeting effect of auxins, as suggested by other work (Byers et al, 1983). It is probable that gibberellins and auxins have a similar mode of action with regard to their anti-russeting properties, as both are known to be able to

increase plant cell wall extensibility (Cleland, 1981). A more successful approach for improving the effect of GA_{4+7} on apple fruit skin finish may be to examine mixtures with other anti-russeting compounds whose mode of action is probably different, such as silicon dioxide or Golclair (Edgerton and Veinbrants, 1979; Steenkamp et al, 1984).

Although the investigation was limited by the lack of sufficient quantities of individual gibberellins, the results indicate that gibberellins differ in their relative effectiveness with regard to the control of russeting and cracking, in agreement with other studies (Wertheim, 1982). GA_4 is apparently the most effective of the gibberellins evaluated and since in contrast to GA_7 it does not inhibit return bloom, this may be the ideal gibberellin for the improvement of skin finish of apple fruit. As yet, however, GA_4 is unavailable to commercial growers and since it is likely to be prohibitively expensive to produce, GA_{4+7} will probably be used for the forseeable future.

The results showed that GA_{4+7} had effects on cell size and shape within the skin tissues of young apple fruit (Chapter 5) and these effects could be related to effects on cell wall extensibility. Young apple fruit sprayed with GA_{4+7} showed increases in the extensibility of skin tissues as measured with an extensometer (Chapter 6) and it seems probable that this change is causally related to the observed effects of GA_{4+7} treatments on russeting and cracking. Further corroborative evidence for such a relationship was provided by the results of the work in which paclobutrazol, a known specific inhibitor of gibberellin biosynthesis, was shown to have the opposite effects to GA_{4+7} on cell morphology, to reduce skin tissue extensibility and to increase the

incidence of russeting and cracking.

Although these results point to increased cell wall extensibility as the mode of action of the anti-russeting properties of GA_{4+7}, further work will be required to determine whether the changes in skin tissue extensibility are due to increased cell wall extensibility or are an indirect effect on cell wall growth. Further work should also endeavour to measure the apple fruit skin tissues at a very early stage of development such as at petal-fall and immediately thereafter. This period has been shown by this and other work to be critical for russet initiation and is the time when GA_{4+7} has to be applied for optimum results. It is unlikely that even a refined and further miniaturised version of the extensometer could be used on skin tissues of fruit much earlier than the stage used here (approximately two weeks after petal-fall and the first GA_{4+7} application) and other techniques will probably be needed in order to do this. One possibility would be the pressure probe technique, which allows the direct measurement of cell wall elasticity and other properties of single cells in plant tissues (Husken et al, 1978). This technique has been used to measure changes in the water relations and elastic properties of apple cortical tissues during the development of the fruit (Steudle and Wieneke, 1985), but these measurements were made on sections of tissue, since it was found to be impossible to introduce the fine tip of the probe across the fruit skin. It would be necessary to find a means of inserting the probe into the epidermal and hypodermal cells if this technique were to be used for evaluating skin tissue extensibility of apple fruit.

The effects of GA_{4+7} and paclobutrazol on russeting, particularly the reversal of the russet inducing properties of the latter by

subsequent applications of the former, suggests that the physiology of the endogenous gibberellins of susceptible apple cultivars is involved in the initiation of the disorder. Eccher (1978, 1986) has attempted to quantify the levels of endogenous gibberellins in apple fruit and to relate these values to the incidence of russeting in particular clones or cultivars. However, the use of bioassay techniques which were employed cannot give accurate quantification of hormone levels nor identify specific hormones (Brenner, 1981). An attempt was made in the present study using modern physico-chemical techniques of hormone analysis, (combined gas chromatography-mass spectrometry with the use of internal standards), to make accurate quantitative and qualitative measurements of endogenous gibberellins in apple fruit, with the aim of relating this to incidence of russeting and cracking in particular clones (Appendix II). These attempts were ultimately unsuccessful and further work is needed using such techniques to clarify the relationship between endogenous gibberellins and the skin finish of apple fruit.

The studies on uptake and translocation of exogenous gibberellins showed that uptake by the fruit was rapid and not affected by concentration, that limited diffusive movement occurred within fruit tissues, and that there was evidence of translocation from leaves to fruit (Chapter 7). There are two main practical implications of these findings. Firstly, since uptake of applied gibberellins by fruit is relatively rapid, then only a few hours of dry weather would be needed after their application for the treatment to be secure. It is apparent, however, that further uptake can occur over a longer period, presumably due to rewetting of the fruit surface by the formation of

dew or in conditions of high relative humidity (Luckwill and Lloyd-Jones, 1962), and this may increase the efficacy of the treatment. Further work, including the identification of gibberellins and possible metabolites in the material recovered from within the fruit tissues, would help to clarify the factors involved in exogenous GA uptake.

Secondly, the fruit must be the main target for GA_{4+7} spray applications if effective control of russeting and cracking is to be achieved. Although there was evidence of limited translocation of gibberellins from leaves to fruit, the experiments involving localised applications of GA_{4+7} showed that when applied only to the leaves there was little, if any, improvement in fruit skin finish.

It is not clear how important an even distribution of the GA_{4+7} spray deposit over the surface of the fruit is, in determining the efficacy of the control of russeting of all regions of the fruit surface. The evidence suggests that there is a limited movement of gibberellin from a treated to an untreated portion of the surface through the fruit tissues, but this may be related to fruit size or more importantly the volume of the fruit concerned. In practical situations it will be important to evaluate the effect of spray volume on the performance of GA_{4+7} spray programmes to improve skin finish. There has been a considerable reduction in the spray volumes used by top-fruit growers in the UK in recent years (Gunn, 1980), and this can lead to less uniform coverage of the target surface with the spray deposit compared to high volume applications. Uneven distribution could affect the performance of the treatment applied, especially in the case of plant growth regulators (Bukovac, 1982). The little work

that has been carried out so far on the effect of spray volume on the control of russeting and cracking with GA_{4+7} has given variable results, with reduced volumes either adversely or not affecting performance (Dijke and Kester, 1983).

A central issue with regard to the commercial application of GA_{4+7} treatments for reducing russeting and cracking is the cost/benefit ratio of such treatments, since to be financially viable it is important that the benefit accrued from the improved skin finish of the apples outweigh the cost of the treatment. Some idea of the costs/benefits involved can be estimated using the following calcuation:

Crop yield (tonne/ha) = A

Price obtained (£/tonne) — Class I = B; Class II = C

Cost of GA_{4+7} treatment (£/ha) = D

Let $E = \frac{D}{B-C}$

Then the percentage increase in Class I grade-out of fruit needed to cover the cost of the treatment = $\frac{E}{A} \times 100$

For example, on a yield of 15 tonne/ha of Cox's Orange Pippin, with prices of £500.00 and £400.00 for Class I and Class II fruit respectively and with the cost of the currently recommended GA_{4+7} spray programme for use in the UK of £136.00/ha (Anon, 1987), then a 6.0% increase in Class I grade-out would be required to cover the cost. Although this calculation does not take into account other costs involved such as manpower inputs, etc, it does show that a relatively small increase in grade-out of fruit is required.

The example given above is not the 'best case', however, since a 15 tonne yield is only just above the national average for Cox's Orange Pippin (MAFF, 1987), while the prices, (equivalent to a 15p/kg difference), are not unreasonable as demonstrated previously (Table 1, Chapter 1). Also, since the rate of GA_{4+7} currently recommended is equivalent to 5 mgl^{-1} and this work has demonstrated that a lower rate may be equally effective, the use of less GA_{4+7} would make the cost effectiveness of such treatments unquestionable. It is interesting to note in this respect that a rate of 2.5 - 5 mgl^{-1} GA_{4+7} is currently recommended for use on Cox's Orange Pippin and Discovery in Holland (Wertheim, 1986b).

The use of plant growth regulators in tree fruit production has not generally fulfilled its original promise (Morgan, 1980), primarily because of unreliability and the variable response obtained with different cultivars, and also because of detrimental side-effects that often accompany the desired response to the treatment (Bukovac, 1985). This work has clearly demonstrated the potential of GA_{4+7} to reduce russeting and cracking of susceptible apple cultivars under UK growing conditions, without any serious risk of detrimental side-effects. It is also clear that it is not the complete answer to the problem. The efficacy of the treatment is greatest when there is a high incidence of russeting and cracking but has little effect when the incidence is low, and occasionally the treatment seems to be without effect as has been reported elsewhere (Martin et al, 1979; Wertheim, 1986b). Gibberellin $A_4 + A_7$ is, however, the most effective anti-russeting material evaluated so far, being superior in its consistency and degree of control than other compounds (Comai and Widmann, 1979; Wertheim, 1980).

Russeting and cracking of apple fruit is a complex phenomenon with numerous factors known to influence its initiation and severity (Walter, 1967; Faust and Shear, 1972a). Growers can do much to prevent the occurrence of the problem and reduce its severity, both by the use of good orchard management (Vogl, 1985) and by growing improved clones of apple which are less susceptible to russeting and cracking, such as Queen Cox (Clark, 1984). There is little possibility, however, of controlling the environmental factors that seem likely to be responsible for the major year to year fluctuations in skin finish that occur, and GA_{4+7} treatments offer the grower the best means of reducing russeting and cracking caused in this way. The work described in this thesis has led to suggestions for the use of GA_{4+7} to improve the skin finish of susceptible apple cultivars, such as Cox's Orange Pippin and Discovery, in the UK (Taylor and Knight, 1985), and reports suggest that growers have obtained good results with such treatments (Anon, 1986). It seems likely that GA_{4+7} treatments will offer the UK grower the best means to improve the skin finish of apples in the foreseeable future.

REFERENCES

ADAMS, P.A., MONTAGUE, M.J., TEPFER, M., RAYLE, D.L., IKUMA, H. and KAUFMAN, P.B. (1975). Effect of gibberellic acid on the plasticity and elasticity of Avena stem segments. Plant Physiology, 56, 757-60.

AGRIHOTHRUDA, V., TRIPATHI, S.C. and VENKATRAMESH, M. (1983). Russeting in apples. Indian Journal of Plant Pathology, 1, 56-61.

ALSTON, F. H. (1973). Apple scion varieties. Preliminary selection. Report of East Malling Research Station for 1972, p.134.

ALSTON, F.H. and WATKINS, R. (1975). Apple breeding at East Malling. Proceedings of Eucarpia Fruit Selection Symposium V. Top Fruit Breeding, Canterbury 1973, 14-29.

ANON. (1986). Safeguards for a finer finish. Grower, 105, 25-27.

ANON. (1987). 'Regulex'. Product Profile. Imperial Chemical Industries PLC.

ASHIZAWA, M., HORIGOME, Y. and CHUJO, T. (1984). Histiological studies on the cause of russet in Golden Delicious apple. Technical Bulletin of Faculty of Agriculture, Kagawa University, 35, 89-99.

BAKER, C.E. (1930). A study of the skin structure of Grimes apple as affected by different types of injury. Proceedings of the American Society for Horticultural Science, 27, 75-81.

BATAL, K.M., WEIGLE, J.L. and FOLEY, D.C. (1970). Relation of stress-strain properties of tomato skin to cracking of tomato fruit. HortScience, 5, 223-4.

BELL, H.P. (1937a). The protective layers of the apple. Canadian Journal of Research, C, 15, 391-402.

BELL, H.P. (1937b). The origin of russeting in the 'Golden Russet' apple. Canadian Journal of Research, C, 15, 560-6.

BOFFELLI, G. and ECCHER, T. (1978). Effetto di trattamenti con gibberelline su alcuni parametri morfologici e sulla partenocarpia dei frutti di 'Golden Delicious'. Rivista della Ortoflorofrutticoltura Italiana, 62, 46-52.

BONDOUX, P., BIDABE, B. and BRIAN, C. (1971). La rugosite (russeting) des pommes. II Stades d'induction. Annales de Phytopathologie, 3, 317-27.

BORSBOOM, O. (1983). PP333 een nieuwe groeiremmer. Fruitteelt, 73, 96-97.

BRENNER, M.L. (1981). Modern methods for plant growth substance analysis. Annual Review of Plant Physiology, 32, 511-38.

BROWN, D.S. and KOCH, E.C. (1962). Stem-end russet of Yellow Newtown apples. Proceedings of the American Society for Horticultural Science, 81, 35-40.

BUKOVAC, M.J. (1973). Foliar penetration of plant growth substance with special reference to tree fruits. Acta Horticulturae, 34, 69-77.

BUKOVAC, M.J. (1982). Low-volume application of plant growth substances to fruit trees. Proceedings XXIst International Horticultural Congress, I, 107-121.

BUKOVAC, M.J. (1985). Plant growth regulators in deciduous tree fruit production: Current status, limitations and future consideration. In: Agricultural Chemicals of the Future. (Hilton, J.E. Ed.) Rowman and Allanheld, Totowa, New Jersey, 75-90.

BUKOVAC, M.J. and NAKAGAWA, S. (1968). Gibberellin-induced asymmetric growth of apple fruits. HortScience, 3, 172-4.

BUKOVAC, M.J., REICHARD, D.L. and WHITMOYER, R.E. (1986). The spray application process: central for the efficient use of growth regulators in tree fruits. Acta Horticulturae, 179, 33-46.

BYERS, R.E. (1978). Chemical thinning of spur 'Golden Delicious' and 'Starkrimson Delicious' with Sevin and Vydate. HortScience, 13, 59-61.

BYERS, R.E., YODER, K.S. and MATTUS, G.E. (1983). Reduction in russeting of 'Golden Delicious' apples with 2,4,5-TP and other compounds. HortScience, 18, 63-65.

CAMPBELL, A.I. (1973). Clonal variation in Cox's Orange Pippin. In: Fruit present and future, Royal Horticultural Society, London, 2, 75-80.

CAMPBELL, A.I. and LACEY, C.N.D. (1975). Induction and selection of mutant forms of fruit plants - Radiation work on other cultivars. Report of Long Ashton Research Station for 1974, p. 18.

CATZEFLIS, J. (1979). Response of apple trees to water stress. Acta Horticulturae, 89, 83-7.

CESARI, A. and FRANCIA, R. (1979). Ricerche sull'azione manifestata da prodatti diversi sulla rugginosita dei frutti "Golden Delicious". Frutticoltura, 41, 10-14.

CHANDLER, F.B. and MASON, J.C. (1942). Russeting of 'Golden Delicious' apples. Proceedings of the American Society for Horticultural Science, 40, 120-2.

CLARK, L.H. (1984). Apple: Trial of clones of Cox's Orange Pippin. Review of Brogdale Experimental Horticulture Station for 1983, 15-16.

CIAMPALINI, M., ROTA, P.A. and SCHULTHAUS, S.DE. (1976). Rugginosita della mele provocata dall'eriofide Aculus schlechtendali. Informatore Agrario, 32, 24243-5.

CLELAND, R.E. (1967). Extensibility of isolated cell walls: measurement and changes during cell elongation. Planta, 74, 197-209.

CLELAND, R.E. (1971). Cell wall extension. Annual Review of Plant Physiology, 22, 197-222.

CLELAND, R.E. (1981). Wall extensibility: hormones and wall extension. In: Encyclopedia of Plant Physiology. N.S. vol. 13B: Plant Carbohydrates II. Extracellular Carbohydrates (Tanner, W. and Loewus, F.A. Eds.). Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 255-73.

CLELAND, R.E. (1984). The Instron technique as a measure of immediate-past wall extensibility. Planta, 160, 514-20.

CLELAND, R.E., THOMPSON, M., RAYLE, D.L. and PURVES, W.K. (1968). Differences in the effects of auxins and gibberellins on wall extensibility of cucumber hypocotyls. Nature, 219, 510-11.

CLEVENGER, J.T. and HAMANN, D.D. (1968). The behaviour of apple skin under tensile loading. Transactions of the American Society of Agricultural Engineers, 11, 34-37.

COBIANCHI, D. and BAGNARA, G.L. (1983). Influenza della GA_{4+7} sul contenimento della rugginosita della mele Golden Delicious. Rivista di Frutticoltura e di Ortofloricoltura, 45, 43-46.

COBIANCHI, D. and FRANCESCONI, A. (1973). E possibile limitare la rugginosita dei frutti "Golden Delicious"? Informatore Agrario,, 29, 14137-8.

COCKBURN, J.T. and SHARPLES, R.O. (1980). A practical guide for assessing starch in Conference pears. Report of East Malling Research Station for 1979, 215-6.

COGGINS, Jr., C.W. (1982). The influence of endogenous growth regulators on rind quality and internal quality of citrus fruits. Proceedings of the International Society of Citriculture, 1981, 1, 214-6.

COMAI, M. and WIDMANN, L. (1979). Reducing epidermal russeting trials on Golden Delicious. Esperienze et Richereche Stazione Sperimentale Agraria Forestale di S. Michele All'adige, 9, 27-32.

CONSIDINE, J.A. and BROWN, K. (1981). Physical aspects of fruit growth: a theoretical analysis of the distribution of surface growth forces in fruit in relation to cracking and splitting. Plant Physiology, 68, 371-6.

COSGROVE, D.J. (1981). Analysis of the dynamic and steady-state responses of growth rate and turgor pressure to changes in cell parameters. Plant Physiology, 68, 1439-46.

COSTA, G., GIULIVO, C. and RAMINA, A. (1983). Influence of growth regulators on apple fruit cracking (cv "Stayman Red"). Acta Horticulturae, 137, 367-74.

CREASY, L.L. (1980). The correlation of weather parameters with russet of 'Golden Delicious' apples under orchard conditions. Journal of the American Society for Horticultural Science, 105, 735-8.

CREASY, L.L. and SWARTZ, H.J. (1981). Agents influencing russet on 'Golden Delicious' apple fruits. Journal of the American Society for Horticultural Science, 106, 203-6.

CRISTOFERI, G. and FILITI, N. (1983). Translocation and accumulation of labelled gibberellic acid into fruitlets and shoot apices of sweet cherry. Acta Horticulturae, 139, 161-70.

CUMMINS, J.N., FORSLINE, P.L. and WAY, R.D. (1977). A comparison of russeting among 'Golden Delicious' subclones. HortScience, 12, 241-2.

CURRY, E.A. and WILLIAMS, M.W. (1983). Promalin or GA_3 increase pedicel and fruit length and leaf size of 'Delicious' apples treated with paclobutrazol. HortScience, 18, 214-5.

DALBRO, K. (1958). Iagttagelser og forsog vedr. skrubben frugt og bladpletter hos Cox's Orange. Tidsskrift for Planteavl, 62, 112-47.

DALZIEL, J. and LAWRENCE, D.K. (1984). Biochemical and biological effects of kaurene oxidase inhibitors, such as paclobutrazol. In: Biochemical Aspects of Synthetic and Naturally Occurring Plant Growth Regulators. (Menhenett, R. and Lawrence, D.K. Eds.). British Plant Growth Regulator Group, Monograph No. 11, 43-57.

DAVIES, L.A. and RAPPAPORT, L. (1975). Metabolism of tritiated gibberellins in d-5 dwarf maize: I. In excised tissues and dwarf and normal plants. Plant Physiology, 55, 620-5.

DECOURTYE, L. (1967). Russet-free sectors on fruits of 'Golden Delicious' apple trees after gamma irradiation. Proceedings of the American Society for Horticultural Science, 91, 73-77.

DENNIS, F.G. (1986). Apple. In: CRC Handbook of Fruit Set and Development. (Monselise, S.P. Ed.). CRC Press, Inc. Boca Raton, Florida, 1-44.

DENNIS, F.G. and EDGERTON, L.J. (1966). Effects of gibberellins and ringing upon apple fruit development and flower bud formation. Proceedings of the American Society for Horticultural Science, 88, 14-24.

DE VRIES, H.A.M.A. (1968). Development of the structure of the normal, smooth cuticle of the apple 'Golden Delicious'. Acta Botanica Neerlandica, 17, 229-41.

DIJKE, J.F. VAN and KESTER, M.W.C. (1983). Kan de GA_{4+7} toepassing beter op de praktijk worden afgestemd? Fruitteelt, 73, 158-60.

DIXON, B., SAGAR, G.R., and SHORROCKS, V.M. (1973). Effect of calcium and boron on the incidence of tree and storage pit in apples of the cultivar Egremont Russet. Journal of Horticultural Science, 48, 403-11.

EASTERBROOK, M.A. and FULLER, M.M. (1986). Russeting of apples caused by apple rust mite Aculus schlechtendali (Acarina: Eriophyidae). Annals of Applied Biology, 109, 1-9.

ECCHER, T. (1975). Influenza di alculi fitormoni sulla rugginosita della "Golden Delicious". Rivista della Ortoflorofrutticoltura Italiana, 59, 246-61.

ECCHER, T. (1978). Russeting of Golden Delicious apples as related to endogenous and exogenous gibberellins. Acta Horticulturae, 80, 381-5.

ECCHER, T. (1983). Control of russeting of Golden Delicious apples by growth regulator treatments. Acta Horticulturae, 137, 375-82.

ECCHER, T. (1986). Russeting and shape of Golden Delicious apples as related to endogenous GA content of fruitlets. Acta Horticulturae, 179, 767-70.

ECCHER, T. and BOFFELLI, G. (1978). Riduzione della rugginosita epidermica della mela Golden Delicious con trattamento di gibberelline. Rivista della Ortoflorofrutticoltura Italiana, 62, 205-11.

ECCHER, T. and BOFFELLI, G. (1981). Effects of dose and time of application of GA_{4+7} on russeting, fruit set and shape of "Golden Delicious" apples. Scientia Horticulturae, 14, 307-14.

ECCHER, T. and CASTELLI, S. (1982). Effetto di trattamenti con GA_{4+7} e GA_{4+7} + BA sulla differenziazione della gemme a fiore nel melo Golden Delicious. Rivista della Ortoflorofrutticoltura Italiana, 66, 313-22.

ECCHER, T. and MAFFI, A. (1986). Treatments for the prevention of Golden Delicious russeting. Acta Horticulturae, 179, 821-2.

EDGERTON, L.J. and VEINBRANTS, N. (1979). Reduction in russeting of "Golden Delicious" apples with silicon dioxide formulations and gibberellins A_{4+7}. HortScience, 14, 40-41.

EDGERTON, L.J., VEINBRANTS, N. and HUTCHINSON, J.F. (1976). Foliar sprays of silicon dioxide - containing compound reduce russeting in "Golden Delicious" apple fruits. HortScience, 11, 508-9.

EGGERT, D.A. and MITCHELL, A.E. (1966). Russeting of Golden Delicious apples as related to soil application of sodium nitrate. Proceedings of the American Society for Horticultural Science, 90, 1-8.

ELFVING, D.C. and ALLEN, O.B. (1987). Effect of gibberellin A_{4+7} applications on "Golden Delicious" fruit russet. Crop Research (Horticultural Research), 27, 11-18.

EPENHUIJSEN, C.W. VAN (1981). Vruchtboomgalmijt (Aculus schlechtendali Nal) een niet te onderschatten plaag in de appelboom. Fruitteelt, 71, 238-41.

FAUST, M. and SHEAR, C.B. (1972a). Russeting of apples, an interpretive review. HortScience, 7, 233-5.

FAUST, M. and SHEAR, C.B. (1972b). Fine structure of the fruit surface of three apple cultivars. Journal of the American Society for Horticultural Science, 97, 351-5.

FENEMORE, P.G. and NORTON, G.A. (1985). Problems of implementing improvements in pest control: a case study of apples in the UK. Crop Protection, 4, 51-70.

FERGUSON, L., WHEATON, T.A., DAVIES, F.S. and ISMAIL, M.A. (1986). ^{14}C-Gibberellic acid uptake, translocation, persistence and metabolism in grapefruit. Journal of the American Society for Horticultural Science, 111, 926-32.

FIRN, R.D. (1986). Growth substance sensitivity: The need for clearer ideas, precise terms and purposeful experiments. Physiologia Plantarum, 67, 267-272.

FISHER, D.F. (1937). York skin crack, hydrochloric acid injury and heat cracking. American Fruit Grower, 57, 11.

FORSLINE, P.L., MUSSELMAN, R.C., KENDER, W.J. and DEE, R.J. (1983). Effects of acid rain on apple tree productivity and fruit quality. Journal of the American Society for Horticultural Science, 108, 70-74.

GARDNER, V.R. and CHRIST, G.E. (1953). Studies on cracking in the Stayman apple. Horticultural News, 34, 2701, 2710-2.

GOLDSCHMIDT, E.E. and GALILI, D. (1981). Fate of ^{14}C-gibberellic acid in senescing on-tree 'Valencia' orange fruit. Journal of the American Society for Horticultural Science, 106, 175-7.

GOLDWIN, G.K. (1984). Factors affecting hormone-assisted setting of Cox's apple. Acta Horticulturae, 149, 161-71.

GOODE, J.E., FULLER, M.M. and HYRYCZ, K.J. (1975). Skin-cracking of Cox's Orange Pippin apples in relation to water stress. Journal of Horticultural Science, 50, 265-9.

GOODE, J.E., HIGGS, K.H. and HYRYCZ, K.J. (1978). Abscisic acid applied to orchard trees of Golden Delicious apple to control water stress. Journal of Horticultural Science, 53, 99-103.

GOODWIN, P.B. (1978). Phytohormones and fruit growth. In: Phytohormones and related compounds: A comprehensive treatise. (Letham, D.S., Goodwin, P.B. and Higgins, T.J.V., Eds.). Volume II, Elsevier/North-Holland, Amsterdam, Oxford, New York, 175-214.

GREEN, P.B. (1976). Growth and cell pattern formation on an axis: critique of concepts, terminology and modes of study. Botanical Gazette, 137, 187-202.

GREENE, D.W. (1984). Microdroplet application of GA_{4+7} + BA: Sites of absorption and effect on fruit set, size and shape of 'Delicious' apples. Journal of the American Society for Horticultural Science, 109, 28-30.

GREENE, D.W. and BUKOVAC, M.J. (1972). Penetration of naphthaleneacetic acid into pears (Pyrus communis L.) leaves. Plant and Cell Physiology, 13, 321-30.

GREENE, D.W., LORD, W.J. and BRAMLAGE, W.J. (1982). Effects of gibberellins A_{4+7} and 6-benzylamino purine on fruit set, fruit characteristics, seed content and storage quality of 'McIntosh' apples. HortScience, 17, 653-4.

GREENBERG, J., GOLDSCHMIDT, E.E., SCHECHTER, S., MONSELISE, S.P. and GALILI, D. (1984). Improving the uptake of gibberellic acid (GA_3) by citrus fruit and leaves. Proceedings of the Eleventh Annual Meeting of the Plant Growth Regulator Society of America, 1984, Boston, 16-24.

GREENHALGH, W.J., GOODLEY, G.L. and MENZIES, R. (1977). Studies of fruit shape in apples: response to gibberellin and cytokinin sprays. Australian Journal of Experimental Agriculture and Animal Husbandry, 17, 505-9.

GREENHAM, D.W.P. (1965). A long-term manurial trial on dessert apple trees. Journal of Horticultural Science, 40, 213-35.

GROCHOWSKA, M.J. (1974). Photolytic decarboxylation of carboxyl-^{14}C-labelled indol-3yl-acetic acid in leaves of apple tree. Journal of Experimental Botany, 25, 638-45.

GUNN, E. (1980). Pesticide application in top fruit - a review. In: Spraying Systems for the 1980's. (Walker, J.O. Ed.). British Crop Protection Council, Monograph No. 24, 253-60.

GUTTRIDGE, C.G. (1962). Inhibition of fruit-bud formation in apple with gibberellic acid. Nature, 196, 1008.

HANKINSON, B. and RAO, V.N.M. (1979). Histological and physical behaviour of tomato skins susceptible to cracking. Journal of the American Society for Horticultural Science, 104, 577-81.

HANKINSON, B., RAO, V.N.M. and SMIT, C.J.B. (1977). Viscoelastic and histological properties of grape skins. Journal of Food Science, 42, 632-5.

HATCH, A.H. (1975). The influence of mineral nutrition and fungicides on russeting of "Goldspur" apple fruit. Journal of the American Society for Horticultural Science, 100, 52-55.

HEDDEN, P. (1987). Gibberellins, In: Principles and Practice of Plant Hormone Analysis, Vol. I. (Rivier, C.L. and Crozier, A. Eds.). Academic Press, London, 9-110.

HEDDEN, P. and GRABE, J.E. (1985). Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of Cucurbita maxima endosperm and Malus pumila embryos. Journal of Plant Growth Regulation, 4, 111-22.

HEDDEN, P. and HOAD, G.V. (1985). Hormonal regulation of fruit growth and development. In: Regulation of Source and Sinks in Crop Plants. (Jeffcoat, B., Hawkins, A.F. and Stead, A.D. Eds). British Plant Growth Regulator Group, Monograph No. 12, 211-24.

HILKENBAUMER, F. (1958). Electronmikroskopische Untersuchungen uber den Aufbau kraftig entwickelter Cuticulae von Apfelfruchen. Zeitschrift Fur Naturforshung, 13B, 666-8.

HOAD, G.V. (1978). The role of seed derived hormones in the control of flowering in apple. Acta Horticulturae, 80, 93-103.

HUSKEN, D., STEUDLE, E. and ZIMMERMAN, U. (1978). Pressure probe technique for measuring water relations of cells in higher plants. Plant Physiology, 61, 158-63.

INGRAM, T.J. and MACMULAN, J. (1986). The quantitative relationship between gibberellin A_1 and internode growth in Pisum sativum. Planta, 168, 414-20.

JACKSON, J.E., PALMER, J.W., PERRING, M.A. and SHARPLES, R.O. (1977). Effects of shade on the growth and cropping of apple trees. III. Effects on fruit growth, chemical composition and quality at harvest and after storage. Journal of Horticultural Science, 52, 267-82.

JONES, R.L. (1973). Gibberellins: their physiological role. Annual Review of Plant Physiology, 24, 571-98.

JONES, R.L. (1982). The role of gibberellins in plant cell elongation. CRC Critical Reviews in Plant Sciences, 1, 23-47.

JONES, R.L. and MACMILLAN, J. (1984). Gibberellins. In: Advanced Plant Physiology. (Wilkins, M.B. Ed.). Pitman Press, London, 21-52.

JOOSSE, M.L. (1982). Discovery: Laten barsten of spuiten? Fruitteelt, 72, 1180-1.

KANBE, K., KON, K. and KUME, Y. (1973). Studies on non-bagging culture of Golden Delicious apples. I. The development and control of russeting on epidermal of Golden Delicious apples. Bulletin of Akita Fruit-Tree Experimental Station, 5, 1-39.

KATSCHNER, E. (1978). Die aussere fruchtqualitat des Golden Delicious lasst sich preisgunstig verbessern! Erwerbsobstbau, 20, 127-32.

KAWAMURA, H., KAMISAKA, S. and MASUDA, Y. (1976). Regulation of lettuce hypocotyl elongation by gibberellic acid. Correlation between cell elongation, stress relaxation properties of the cell wall and wall polysaccharide content. Plant Cell Physiology, 17, 23-34.

KIRBY, A.H.M. and BENNETT, M. (1967). Susceptibility of apple and pear varieties to damage by certain organic fungicides. Journal of Horticultural Science, 42, 117-31.

KIRKWOOD, P.S. and MACMILLAN, J. (1982). Gibberellins A60, A61 and A62: partial synthesis and natural occurrence. Journal of the Chemical Society Perkin Transactions, 1, 689-97.

KLACKLE, F. (1978). Apple production in Japan. Compact Fruit Tree, 11, 80-82.

KNEE, M., SMITH, S.M. and JOHNSON, D.S. (1983). Comparison of methods for estimating the onset of the respiration climacteric in unpicked apples. Journal of Horticultural Science, 58, 521-6.

KNIGHT, J.N. and WEBSTER, A.D. (1986). Translocation of gibberellic acid and of fruit setting/retaining stimuli in Conference pear, Victoria plum and Early Rivers cherry. Journal of Horticultural Science, 61, 191-200.

KNUTH, D. and STOSSER, R. (1987). Vergleich der sonnen-und schattenseite von apfelfruchten I. kutikula, epidermiszelgrobe und oberflachenwachse. Gartenbauwissenschaft, 52, 49-57.

KOLATTUKUDY, P.E. (1980). Biopolyester membranes of plants: Cutin and Suberin. Science, 208, 990-1000.

KOSHIOKA, M., TAYLOR, J.S., EDWARDS, G.R., LOONEY, N.E. and PHARIS, R.P. (1985). Identification of gibberellins A_{19} and A_{20} in vegetative tissue of apple. Agricultural and Biological Chemistry, 49, 1223-6.

KREMER, F.W. (1967). Influence of pesticides on russeting of Golden Delicious. Pflanzenschutz-Nachrichten Bayer, 20, 629-43.

LACEY, C.N.D. (1982). Radiation-induced mutants of apple. Report of Long Ashton Research Station for 1980, 192-207.

LACEY, C.N.D. and SPARKS, T.R. (1982). Induction and selection of mutant apples - Off-Station trials in commercial orchards, 2/ Queen Cox Selection Orchard, Blackmoor Estate, Hampshire. Report of Long Ashton Research Station for 1980, p.25.

LAPINS, K.O. (1971). Mutants of Golden Delicious apple induced by ionizing radiation. Canadian Journal of Plant Science, 51, 123-31.

LEVER, B.G. (1986). "Cultar" - a technical overview. Acta Horticulturae, 179, 456-66.

LINK, H. (1973). Effect of fruit thinning on some components of fruit quality in apples. Acta Horticulturae, 34, 445-8.

LINSKENS, H.F. and GELISSEN, A. (1966). Die Natur der Rauschaligkeit bei Fruchten der Apfelsorte "Golden Delicious". Phytopathalogische Zeitschrift, 57, 1-7.

LONG, S.M. (1980). Cuticle development and incidence of russet on "Golden Delicious" apple as influenced by subclone susceptibility and shelters. M.Sc. Thesis, Michigan State University.

LOONEY, N.E. (1979). Some effects of gibberellins A_{4+7} plus benzyladenine on fruit weight, shape, quality, Ca content, and storage behaviour of "Spartan" apple. Journal of the American Society for Horticultural Science, 104, 389-91.

LOONEY, N.E. and PHARIS, R.P. (1986). Gibberellins and reproductive development of tree fruits and grapes. Acta Horticulturae, 179, 59-71.

LOONEY, N.E., KAMIENSKA, A., LEGGE, R.L. and PHARIS, R.P. (1978). Metabolism of ^{3}H gibberellin A_4 in relation to flower initiation in apple. Acta Horticulturae, 80, 105-14.

LOONEY, N.E., PHARIS, R.P. and NOMA, M. (1985). Promotion of flowering in apple trees with gibberellin A_4 and C-3 epi-gibberellin A_4. Planta, 165, 292-4.

LOTT, R.V. (1957). The quality and keepability of "Golden Delicious" apples having russet bands caused by a frost. Proceedings of the American Society for Horticultural Science, 69, 56-64.

LOTTER, J.de V. and VAN ZYL, E.J. (1964). Growth and stem-end russeting of Ohenimuri apples in relation to their position in the cluster. Decidious Fruit Grower, 14, 302-5.

LUCKWILL, L.C. (1968). The effect of certain growth regulators on growth and apical dominance of young apple trees. Journal of Horticultural Science, 43, 91-101.

LUCKWILL, L.C. and LLOYD-JONES, C.P. (1962). The absorption, translocation and motabolism of 1-naphthaleneacetic acid applied to apple leaves. Journal of Horticultural Science, 37, 190-206.

MAFF (Ministry of Agriculture Fisheries and Food, London), (1973). EEC standards for fresh apples and pears. HMSO, London.

MAFF (Ministry of Agriculture Fisheries and Food, London), (1987). Basic horticultural statistics for the United Kingdom. Calendar and Crop Years 1977-1986. Statistics (Agricultural Commodities) Division, Branch B, Great Westminster House, London.

MARCELLE, R. and SIRONVAL, C. (1963). Effect of gibberellic acid on flowering of apple trees. Nature, 197, 405.

MARTIN, C.M., TYLER, R.H. and NISHIJIMA, C. (1979). Apple russet on Yellow Newtown Pippin. California Agriculture, 33, 13.

McCOMB, A.J. (1964). The stability and movement of gibberellic acid in pea seedlings. Annals of Botany, 28, 669-87.

McLAUGHLIN, J.M. and GREENE, D.W. (1984). Effects of BA, GA_{4+7} and daminozide on fruit set, fruit quality, vegetative growth, flower initiation, and flower quality of "Golden Delicious" apple. Journal of the American Society for Horticultural Science, 109, 34-39.

MEADOR, D.B. (1977). Reducing russet on "Golden Delicious" apples with silicon dioxide formulation foliage sprays. HortScience, 12, 504-5.

MEADOR, D.B. and TAYLOR, B.H. (1987). Effect of early season foliar sprays of GA_{4+7} on russeting and return bloom of "Golden Delicious" apple. HortScience, 22, 412-5.

MEYER, A. (1944). A study of the skin structure of Golden Delicious apples. Proceedings of the American Society for Horticultural Science, 45, 105-10.

MEYER, R.H. (1982). Fruit thinning and russeting effects of oxamyl on apple. HortScience, 17, 658-9.

MILLER, R.H. (1982). Apple fruit cuticles and the occurrence of pores and transcuticular canals. Annals of Botany, 50, 355-71.

MIZUNO, N. and TAKAHASHI, S. (1978). Russeting on the epidermis of apple fruit caused by early infection with Alternaria mali. Bulletin of the Akita Fruit-Tree Experiment Station, 10, 45-52.

MONIN, A. (1961). The effects of rootstock on the external quality of Cox apples. Revue De L'Agriculture Bruxelles, 14, 631-9.

MONTGOMERY, H.B.S. (1959). Russeting and cracking of Cox's Orange Pippin apples. Report of East Malling Research Station for 1958, 163-4.

MORGAN, P. (1980). Synthetic growth regulators: potential for development. Botanical Gazette, 141, 337-46.

NAKAGAWA, S., BUKOVAC, M.J., HIRATA, N. and KUROAKA, H. (1968). Morphological studies of gibberellin-induced parthenocarpic and asymmetric growth in apple and Japanese pear fruits. Journal of the Japanese Society for Horticultural Science, 37, 9-19.

NOGA, G.J. and BUKOVAC, M.J. (1986). Impact of surfactants on fruit quality of "Schattenmorelle" sour cherries and "Golden Delicious" apples. Acta Horticulturae, 179, 771-7.

NOTEBOOM, M. (1976). Vochtvoorziening en veruwing bij Golden Delicious. Fruitteelt, 66, 890-1.

OLSON, A.C., BONNER, J. and MORRE, D.J. (1965). Force extension analysis of Avena coleoptile cell walls. Planta, 66, 127-33.

OOSTEN, H.J. VAN, MEIJNEKE, C.A.R. and PEERBOOMS, H. (1982). Growth, yield and fruit quality of virus-infected and virus-free Golden Delicious apple trees, 1968-1982. Acta Horticulturae, 130, 213-20.

PALMITER, D.H. (1944). Relation of spray materials to russeting of Delicious and Golden Delicious apples. Proceedings of the American Society for Horticultural Science, 45, 113-18.

PERRING, M.A. (1979). The effect of environment and cultural practices on calcium concentration in the apple fruit. In: Communications in Soil Science and Plant Analysis, (Shear, C.B. Ed.). Marcel Dekker Inc. New York, 279-93.

PERRING, M.A. (1986). Incidence of bitter pit in relation to the calcium content of apples: problems and paradoxes, a review. Journal of the Science of Food and Agriculture, 37, 591-606.

PHARIS, R.P. and KING. R.W. (1985). Gibberellins and reproductive development in seed plants. Annual Review of Plant Physiology, 36, 517-68.

PHINNEY, B.O. (1984). Gibberellin A_1, dwarfism and the control of shoot elongation in higher plants. In: The Biosynthesis and Metabolism of Plant Hormones. (Crozier, A. and Hillman, J.R. Eds.). Society for Experimental Biology Seminar Ser. No. 23, Cambridge University Press, 17-45.

PRATT, C. (1972). Periderm development and radiation stability of russet-fruited sports of apple. Horticultural Research, 12, 5-12.

PROBINE, M.C. and PRESTON, R.D. (1961). Cell growth and the structure and mechanical properties of the cell wall in internodal cells of Nitella opaca. I. wall structure and growth. Journal of Experimental Botany, 12, 261-80.

RADEMACHER, W., JUNG, J., GRAEBE, J.E. and SCHWENEN, L. (1984). On the mode of action of tetcylacis and triazole growth retardants. In: Biochemical Aspects of Synthetic and Naturally Occurring Plant Growth Regulators. (Menhenett, R. and Lawrence, D.K. Eds.). British Plant Growth Regulator Group, Monograph No. 11, 1-11.

RAMIREZ, H. and HOAD, G.V. (1978). Effects of succinic acid 2,2-dimethylhyrazide (SADH) and hormones on flower initiation in apple. In: The Effect of Interactions between Growth Regulators on Plant Growth and Yield, British Plant Growth Regulator Group, Monograph No. 2, 37-47.

RICHARDSON, P.J., WEBSTER, A.D. and QUINLAN, J.D. (1986). The effect of paclobutrazol sprays with or without the addition of surfactants on the shoot growth, yield and fruit quality of the apple cultivars Cox and Suntan. Journal of Horticultural Science, 61, 439-46.

ROACH, F.A. (1980). Apple production in England - its history from Roman times to the present day. Report of Long Ashton Research Station for 1979, 216-33.

ROBINSON, J. and WINFIELD, A.L. (1981). Tests of acaracides against apple rust mite. Tests of Agrochemicals and Cultivars (Annals of Applied Biology, 97, Supplement No. 2), 18-19.

ROOIJEN, W.J. VAN (1983). Vermindering vruchtverruwing bij appel door toepassing van berelex GA$_{4/7}$. Fruitteelt, 73, 398-9.

RUI, D. (1975). Utilita dei trattamenti con Golclair contro la rugginosita delle mele. Informatore Agrario, 31, 21145-6.

SACHS, R.M. and WEAVER, R.J. (1968). Gibberellin and auxin-induced berry enlargement in Vitis vinifera L. Journal of Horticultural Science, 43, 185-95.

SAMUELSON, T.J. and HOLLAND, D.A. (1983). The effect of size and sub-sample on mineral analysis values of apple fruits. Journal of the Science of Food and Agriculture, 34, 198-202.

SANSAVINI, S. and BASSI, D. (1977). Clonal selection, fertility and fruit quality of Golden Delicious. Acta Horticulturae, 75, 73-84.

SCHOLTENS, A. and BOOTSMA, J.J. (1981). Gibberellinen tegen vruchtverruwing I. Proefervaringen 1980 en advies 1981. Fruitteelt, 71, 507-9.

SCHUMACHER, R and FANKHAUSER, F. (1967). Wirkung chemischer ausdunnungsmittel bei Golden Delicious auf berostung, fruchtansatz und qualitat. Schweizerische Zeitschrift fur Obst-und Weinbau, 103, 290-300, 315-24.

SCHUMACHER, R., FANKHAUSER, F. and STADLER, W. (1977). Fruchtausdunnung bei der sorte Golden Delicious. Schweizerische Zeitschrift fur Obst-und Weinbau, 113, 188-92.

SHARPLES, R.O. and JOHNSON, D.S. (1986). Effects of some growth regulators on the ripening and storage quality of apples and pears. Acta Horticulturae, 179, 721-30.

SHARPLES, R.O. and LITTLE, R.C. (1970). Experiments on the use of calcium sprays for bitter pit control in apples. Journal of Horticultural Science, 45, 49-56.

SHUTAK, V. and SCHRADER, A.L. (1948). Factors associated with skin-cracking of York Imperial apples. Proceedings of the American Society for Horticultural Science, 51, 245-57.

SIMONS, R.K. (1957). Frost injury on Golden Delicious apples - morphological and anatomical characteristics of russeted and normal tissue. Proceedings of the American Society for Horticultural Science, 69, 48-55.

SIMONS, R.K. (1960). Developmental changes in russet sports of "Golden Delicious" apples - morphological and anatomical comparison with normal fruit. Proceedings of the American Society for Horticultural Science, 80, 79-89.

SIMONS, R.K. (1962). Spontaneous russet sports of Golden Delicious apples - morphological and anatomical comparison with normal fruit. Proceedings of the American Society for Horticultural Science, 80, 79-89.

SIMONS, R.K. (1965). The origin of russeting in russet sports of the Golden Delicious apple. Horticultural Research, 5, 101-6.

SIMONS, R.K. (1969). Tissue response of young developing apple fruits to freeze injury. Journal of the American Society for Horticultural Science, 94, 376-82,

SIMONS, R.K. and DOLL, C.C. (1976). Morphological and anatomical response of apples to a late spring frost in relation to stage of fruit development. Journal of the American Society for Horticultural Science, 101, 315-20.

SIMONS, R.K. and LOTT, R.V. (1963). The morphological and anatomical development of apples injured by a late spring frost. Proceedings of the American Society for Horticultural Science, 83, 88-100.

SIRONVAL, C. and CLIJSTERS, H. (1962). Sur la nature de la rugosite chez la pomme Cox's Orange. Bulletin de la Societe Royale de Botanique de Belgique, 94, 35-44.

SKENE, D.S. (1962). Fruit skin structure in some tree fruits, with special reference to russeting of apples. Ph.D. Thesis, London University.

SKENE, D.S. (1965). Cracking and russeting in apple fruits. Report of East Malling Research Station for 1964, 99-101.

SKENE, D.S. (1966). The distribution of growth and cell division in the fruit of Cox's Orange Pippin. Annals of Botany, 30, 493-512.

SKENE, D.S. (1980a). Growth stresses during fruit development in Cox's Orange Pippin apples. Journal of Horticultural Science, 55, 27-32.

SKENE, D.S. (1980b). Effect of supplementary dimethoate sprays on russeting of Golden Delicious apples. Journal of Horticultural Science, 55, 113-17.

SKENE, D.S. (1981). Wound healing in apple fruits: the anatomical response of Cox's Orange Pippin at different stages of development. Journal of Horticultural Science, 56, 145-53.

SKENE, D.S. (1982). The development of russet, rough russet and cracks on the fruit of Cox's Orange Pippin during the course of the season. Journal of Horticultural Science, 57, 165-74.

SKEENKAMP, J., VAN ZYL, H.J. and WESTRAAD, I. (1984). A preliminary evaluation of various chemical substances for the control of calyx-end russeting in Golden Delicious apples. Journal of Horticultural Science, 59, 501-5.

STEMBRIDGE, G.E. (1973). Effect of growth regulators on the size and shape of fruits. Acta Horticulturae, 34, 435-40.

STEMBRIDGE, G.E. and MORELL, G. (1972). Effect of gibberellins and 6-benzyladenine on the shape and fruit set of "Delicious" apples. Journal of the American Society for Horticultural Science, 97, 464-7.

STEUDLE, E. and WIENEKE, J. (1985). Changes in water relations and elastic properties of apple fruit cells during growth and development. Journal of the American Society for Horticultural Science, 110, 824-9.

STUART, D.A. and JONES, R.L. (1977). Roles of extensibility and turgor in gibberellin and dark stimulated growth. Plant Physiology, 59, 61-68.

STUBBINGS, W.A.K. and STRYDOM, D.K. (1965). Russeting of Golden Delicious apples in the Elgin area. Deciduous Fruit Grower, 15, 149-51.

TAYLOR, B.K. (1975). Reduction of apple skin russeting by gibberellin GA_{4+7}. Journal of Horticultural Science, 50, 169-72.

TAYLOR, B.K. (1978). Effect of gibberellin sprays on fruit russet and tree performance of Golden Delicious apple. Journal of Horticultural Science, 53, 167-9.

TAYLOR, D.R. and KNIGHT, J.N. (1985). Making up with gibberellins for a finer skin. Grower, 103, 23-25.

TAYLOR, D.R., FULLER, M.M. and BROOKFIELD, M. (1985). Effects of plant growth regulators on apple skin morphology in relation to the control of russeting. Report of East Malling Research Station for 1984, 106-7.

TETLEY, U. (1930). A study of the anatomical development of the apple and some observations on the "Pectic Constituents" of the cell walls. The Journal of Pomology and Horticultural Science, 8, 153-72,

TETLEY, U. (1931). The morphology and cytology of the apple fruit, with special reference to Bramley's Seedling variety. The Journal of Pomology and Horticultural Science, 9, 278-97.

TOPPING, A.J. (1981). A recording laboratory penetrometer for fruit. Journal of Agricultural Engineering Research, 26, 179-83.

TROMP, J. (1973). The interaction of growth regulators and tree orientation on fruit-bud formation. Acta Horticulturae, 34, 185-8.

TROMP, J. (1982). Flower-bud formation in apple as affected by various gibberellins. Journal of Horticultural Science, 57, 277-82.

TROMP, J. (1987). Growth and flower-bud formation in apple as affected by paclobutrazol, daminozide, and tree orientation in combination with various gibberellins. Journal of Horticultural Science, 62, 433-40.

TUKEY, H.B. and YOUNG, J.O. (1942). Gross morphology and histology of developing fruit of the apple. Botanical Gazette, 104, 3-25.

TUKEY, L.D. (1959). Observations on the russeting of apples growing in plastic bags. Proceedings of the American Society for Horticultural Science, 74, 30-9.

VAN VOLKENBURGH, E., HUNT, S. and DAVIES, W.J. (1983). A simple instrument for measuring cell-wall extensibility. Annals of Botany, 51, 669-72.

VARGA, A. (1969). Effects of growth regulators on fruit set and June drop of pears and apples. Netherlands Journal of Agricultural Science, 17, 229-33.

VEINBRANTS, N. and MILLER, P. (1981). Promalin improves the shape of Delicious apples in Victoria. Australian Journal of Experimental Agriculture and Animal Husbandry, 21, 623-30.

VERNER, L. (1935). A physiological study of cracking in Stayman Winesap apples. Journal of Agricultural Research, 51, 191-222.

VERNER, L. (1938). Histology of apple fruit tissue in relation to cracking. Journal of Agricultural Research, 57, 813-24.

VOGL, M. (1985). Zur fruchtberostung bei der apfelsorte "Gelber Kostlicher". Gartenbau, 32, 84-85.

VOISEY, P.W., LYALL, L.H. and KLOEK, M. (1970). Tomato skin strength - its measurement and relation to cracking. Journal of the American Society for Horticultural Science, 95, 485-8.

WALTER, T.E. (1967). Russeting and cracking in apples: a review of world literature. Report of East Malling Research Station for 1966, 83-95.

WATANABE, S. (1969). Histological studies on the cause of russet in apples. Bulletin Yamagata University Agricultural Science, 5, 823-936.

WEAVER, R.J. and POOL, R.M. (1971). Thinning "Tokay" and "Zinfandel" grapes by bloom sprays of gibberellin. Journal of the American Society for Horticultural Science, 96, 820-2.

WERTHEIM, S.J. (1971). The drop of flowers and fruits in apple, with special reference to the June drop of Cox's Orange Pippin and its control with growth regulators. Mededelingen Landbouwhogeschool, Wageningen, 71(17), 73pp.

WERTHEIM, S.J. (1973). Fruit set and June drop in Cox's Orange Pippin apple as affected by pollination and treatment with a mixture of gibberellins A_4 and A_7. Scientia Horticulturae, 1, 85-105.

WERTHEIM, S.J. (1980). Nieuwe middelen tegen vruchtverruwing. Fruitteelt, 70, 224-5.

WERTHEIM, S.J. (1982). Fruit russeting in apple as affected by various gibberellins. Journal of Horticultural Science, 57, 283-88.

WERTHEIM, S.J. (1986a). Chemical thinning of Golden Delicious apple with NAAm and/or carbaryl in combination with a spreader and the anti-russeting agent GA_{4+7}. Acta Horticulturae, 179, 659-66.

WERTHEIM, S.J. (1986b). Gibberellinen tegen vruchtverruwing. Fruitteelt, 76, 548-50.

WESTERLAKEN, J. (1982). Naar betere kwaliteit met Berelex $GA_{4/7}$. Fruitteelt, 72, 498-9.

WESTWOOD, M.N. (1962). Seasonal changes in specific gravity and shape of apple, pear and peach fruits. Proceedings of the American Society for Horticultural Science, 80, 90-96.

WESTWOOD, M.N. (1978). Fruit growth and thinning. In: Temperate Zone Pomology. W.H. Freeman and Co., San Francisco, 199-219.

WESTWOOD, M.N. and BJORNSTAD, H.O. (1968). Effects of gibberellin A3 on fruit shape and subsequent seed dormancy of apple. HortScience, 3, 19-20.

WESTWOOD, M.N. and BLANEY, L.T. (1963). Non-climatic factors affecting the shape of apple fruits. Nature, 200, 802-3.

WILLIAMS, M.W. (1979). Chemical thinning of apples. Horticultural Reviews, 1, 270-300.

WILLIAMS, M.W. and STAHLY, E.A. (1969). Effect of cytokinins and gibberellins on shape of "Delicious" apple fruits. Journal of the American Society for Horticultural Science. 94, 17-19.

YOGARATNAM, N. and JOHNSON, D.S. (1982). The application of foliar sprays containing nitrogen, magnesium, zinc and boron to apple trees. II. Effects on the mineral composition and quality of the fruit. Journal of Horticultural Science, 57, 159-64.

ZORBRIST, L. (1962). Apple mildew (Podosphaera leucotricha) as a cause of russeting apples. Proceedings of the British Insecticide and Fungicide Conference, 1961, Brighton, 1, 237-8.

ZSCHOKKE, A. (1897). Uber dem bau der haut und die ursachen der verschiedenen haltbarkeit unserer kernobstfruchte. Landwirt Jahrb der Schweiz, 11, 153-97.

ZWEIG, G., YAMAGUCHI, S. and MASON, G.W. (1961). Translocation of 14C-gibberellin in red kidney bean, normal corn and dwarf corn. Advances in Chemistry Series, 28, 122-34.

FURNEAUX, B.S. (1954). The soils of East Malling Research Station. Report of East Malling Research Station for 1953, 60-82.

APPENDIX I

SPRAYING DATES FOR TIMING/FREQUENCY EXPERIMENT - 1983

Treatment	Spraying Date	Treatment	Spraying Date
1 F2 I1 T1	10/5,17/5	19 F4 I2 T1	10/5,24/5,7/6,21/6
2 F2 I1 T2	13/5,20/5	20 F4 I2 T2	13/5,27/5,10/6,24/6
3 F2 I1 T3	24/5,31/5	21 F4 I2 T3	24/5,7/6,21/6,5/7
4 F2 I1 T4	31/5,7/6	22 F4 I2 T4	31/5,14/6,28/6,12/7
5 F2 I1 T5	7/6,14/6	23 F4 I2 T5	7/6,21/6,5/7,19/7
6 F2 I1 T6	14/6,21/6	24 F4 I2 T6	14/6,28/6,12/7,26/7
7 F2 I2 T1	10/5,24/5	25 F6 I1 T1	10/5,17/5,24/5,31/5,7/6,14/6
8 F2 I2 T2	13/5,27/5	26 F6 I1 T2	13/5,20/5,27/5,3/6,10/6,17/6
9 F2 I2 T3	24/5,7/6	27 F6 I1 T3	24/5,31/5,7/6,14/6,21/6,28/6
10 F2 I2 T4	31/5,14/6	28 F6 I1 T4	31/5,7/6,14/6,21/6,28/6,5/7
11 F2 I2 T5	7/6,21/6	29 F6 I1 T5	7/6,14/6,21/6,28/6,5/7,12/7
12 F2 I2 T6	14/6,28/6	30 F6 I1 T6	14/6,21/6,28/6,5/7,12/7,19/7
13 F4 I1 T1	10/5,17/5,24/5,31/5	31 F6 I2 T1	10/5,24/5,7/6,21/6,5/7,19/7
14 F4 I1 T2	13/5,20/5,27/5,3/6	32 F6 I2 T2	13/5,27/5,10/6,24/6,8/7,22/7
15 F4 I1 T3	24/5,31/5,7/6,14/6	33 F6 I2 T3	24/5,7/6,21/6,5/7,19/7,2/8
16 F4 I1 T4	31/5,7/6,14/6,21/6	34 F6 I2 T4	31/5,14/6,28/6,12/7,26/7,9/8
17 F4 I1 T5	7/6,14/6,21/6,28/6	35 F6 I2 T5	7/6,21/6,5/7,19/7,2/8,16/8
18 F4 I1 T6	14/6,21/6,28/6,5/7	36 F6 I2 T6	14/6,28/6,12/7,26/7,9/8,23/8

Key:	Timing of initial application	Number of spray applications	Spray interval days
	T1 first flower	F2 2	I1 7
	T2 full bloom	F4 4	I2 14
	T3 80% petal-fall	F6 6	
	T4 7 days after T3		
	T5 14 days after T3		
	T6 21 days after T3		

APPENDIX II

A DESCRIPTION OF THE TECHNIQUES INVOLVED IN THE QUANTITATIVE AND QUALITATIVE ANALYSIS OF THE ENDOGENOUS GIBBERELLINS IN CLONES OF QUEEN COX

INTRODUCTION

To date, a total of 23 endogenous gibberellins have been unequivocally indentified by gas chromatography - mass spectrometry (GC-MS) in immature apple seed tissues, including GA_4 and GA_7 (Hoad, 1978; Kirkwood and MacMillan, 1982; Hedden and Hoad, 1985). Interestingly, GA_{19}, the major GA found in vegetative tissue (Koshioka et al, 1985), has not been characterised from developing seeds. Eccher (1978, 1986), has related variation in the incidence of russeting to variations in the levels of endogenous gibberellins found in the fruit of Golden Delicious but his measurements of GA levels involved bioassay techniques, which cannot give accurate quantitative results (Brenner, 1981).

The work described here was an attempt to use modern physico-chemical techniques of hormone analysis to measure the endogenous gibberellin content of fruit from trees of clones of Queen Cox which had a history of known wide variation in the incidence and severity of russet and cracking that developed each year. The clones involved had arisen from the programme of work at Long Ashton Research Station to develop improved forms of apple varieties by the production of mutations, by subjecting dormant vegetative material to ionizing radiation (Lacey, 1982).

MATERIALS AND METHODS

The trees of Queen Cox used in this work were situated in an orchard containing 5000 trees planted at Blackmoor Estates, Liss, Hampshire in 1975 and which contained 3836 mutants produced by irradiating Queen Cox material with gamma rays from a ^{60}Co source (Campbell and Lacey, 1974; Lacey and Sparks, 1982). Two populations with six trees in each were chosen from records of previous cropping performance to give as wide a range as possible of inherent skin finish characteristics including clones with superior skin finish and others prone to develop russeting and cracking (T R Sparks, personal communication).

Samples of fruit were taken at random from all parts of tree, from all the trees in each population, starting on 26 May 1983 (petal-fall) and then at weekly intervals until 30 June. A total of approximately 90-100 g fresh weight was sampled from each population on each sampling date, the samples being immediately plunged into liquid nitrogen and then transported to East Malling Research Station. The samples were stored at -18^{o} until required for extraction.

Extraction and Purification

Sub-samples were taken from each sample, approximately 20 g being used for each extraction. The frozen material was homogenised in cold ($\sim 5^{o}C$) 80% methanol containing 20 mgl^{-1} butylated hydroxytoluene as an antioxidant. At this stage 1µg of [17-$^{2}H_{2}$] GA_{4} synthesized from GA_{4} 17-nor-16-one using the Wittig reaction (see Hedden, 1987), was added to the homogenate as an internal standard. After extraction, the extract was filtered (Whatman No. 5 filter paper) under vacuum and

washed with 2 x 50 cm^3 methanol. The combined extract and washings were reduced at 30°C to the aqueous phase under reduced pressure on a rotary evaporator (RFE). This was then subjected to solvent partioning as shown in Figure 5.

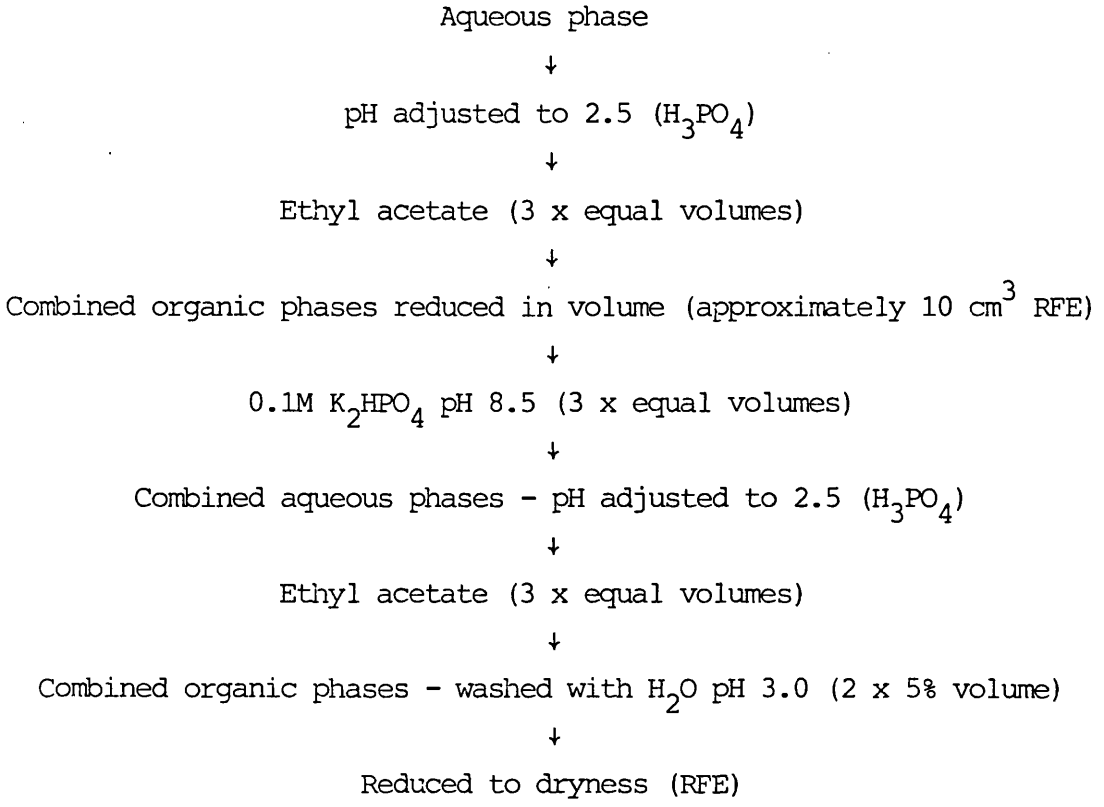


Figure 5. Flow diagram outlining solvent partioning procedure.

The extract was dissolved in 5 cm^3 of 0.1M K_2HPO_4 at pH 8.5 and poured onto a column containing 0.5 g of polyvinylpyrrolidone (PVP30-100 mesh), which had been primed with 50 cm^3 0.1M KH_2PO_4 pH 3.0. The original container was rinsed with the same buffer (2 x 5 cm^3) and poured onto the column. The pH of the combined eluates was adjusted to 3.0 with H_3PO_4 and the solution loaded onto a 'Sep-Pak' C18 silica cartridge (Walters Associates) which had been primed with methanol (5 cm^3) and 5% aqueous acetic acid (2 x 5 cm^3). The cartridge was washed

with 5 cm^3 of 5% aqueous acetic acid, followed by 5 cm^3 of double distilled H_2O, and the gibberellins were recovered from the silica by eluting with 5 cm^3 of 80% methanol. The eluate was then reduced to dryness with a 'Speed-Vac' centrifugal vacuum concentrator.

The extract was further purified by high performance liquid chromatography (HPLC) using a Kontron HPLC system 600 liquid chromatograph. The sample was taken up in 30µl methanol and then 20µl was injected into a 250 x 5 mm i.d. reverse phase RP8 column. The gradient controller was set to deliver 20% methanol ~ 10 min, a 10 min linear gradient to 80% methanol which was held for ~ 5 min and then a 5 min linear gradient to 20% methanol. The flow rate was 1 cm^3 min^{-1}, the elutent being collected between 20 and 30 min after injection. The elutent was then reduced to dryness with a 'Speed-Vac' centrifugal vacuum concentrator.

The dried sample was dissolved in 100µl of methanol and then etheral diazomethane was added until the yellow colour of the solution remained. This was left to stand for ~10 min, a drop of glacial acetic acid was added to destroy unreacted diazomethane and the solution taken to dryness in the vacuum concentrator. The samples were transferred to glass ampoules using dry dichlomethane (2 x 50 µl), and the solvent removed in the vacuum concentrator. The trimethylsilyl ethers (TMS) of the gibberellin methyl esters were prepared by adding 100 µl of N,O-bis (trimethylsilyl)acetamide (Tri-Sil/BSA; Pierce Chemical Co.) to the extract and heating the sealed ampoule at 90°C for 30 min.

Capillary column GC-MS

Combined GC-MS was performed as described by Hedden (1987), on a

VG 1212 computerized mass spectrometer (VG Analytical, Wythenshawe, Manchester, UK) coupled to a DANI-3800 HR gas chromatograph (Kontron Instruments, St Albans, Herts, UK). The SE-30 WC0T quartz capillary column (25 m x 0.2 mm i.d.) was coupled directly to the ion source, at an interface temperature of 200°C. The GC injector temperature was 200°C and the He carrier-gas inlet pressure 1.5 bar. Samples (1-2µl) were injected at 50C without splitting, after 30s the split (50:1) was opened and 30s later the column was heated ballistically to 240°C and then programmed at 5°C min^{-1} to 300°C. Data was collected from 240°C. The MS electron energy used was 70 eV, the emission current 100 µA and the source temperature 200°C. For quantitation by multiple ion monitoring (MIM) the VG Foreground/Background Selected Ion Recording (FBSIR) software was used to monitor at 1000 resolving power responses to ions at m/z 222, 282, 284, 286, 416, 418, 420 and 506. These ions are characterisic for the major GAs identified in apple seed and [17-2 H_2] GA_4, the interval standard.

All solvents were redistilled before use.

RESULTS AND DISCUSSION

Unfortunately the samples as prepared were still not pure enough for GC-MS and there was considerable interference at the peaks of interest by co-eluting impurities. There was insufficient time available to adopt the purification procedure to remove these impurities. This problem with the purity of the extract is not uncommon with the measurement of endogenous gibberellins in higher plant tissues (eg Rademacher et al, 1984). The problem was increased because whole fruit tissues were used, rather than seeds only and this would have added to the impurities obtained in the samples. If more

time had been available, extraction of the seeds from the fruit would have been appropriate, with extraction of seeds and flesh as separate samples.

Appendix III

BACKGROUND INFORMATION RELATING TO THE FIELD EXPERIMENTS

GENERAL

As mentioned in the general materials and methods, p55, trees were assigned to blocks in each experimental layout by means of the number of fruit buds counted before flowering. An excess number of trees were measured in this way, with very poor and very large/vigorous trees being discarded, the trees used in the experiments being chosen so as to give as uniform material as possible. No measurement of tree size was used in the blocking procedure, although the trees within blocks were generally very uniform with regard to size, with atypical trees having been eliminated as described above.

SOILS

The soils of the orchards used at East Malling Research Station for the field experiments are as follows, all having been described by Furneaux (1954).

Chapter 2

Cox's Orange Pippin - 1983	Malling series
Cox's Orange Pippin - 1984	Barming series + Medway series
Golden Delicious - 1983	Barming series + Malling series
Discovery - 1984(1)	Twisden series + Bradbourne series
Discovery - 1984(2)	Malling series + Barming series

Chapter 3

Cox's Orange Pippin - 1983	Malling series + Langley series
Cox's Orange Pippin - 1984	Barming series

Chapter 4

Cox's Orange Pippin - 1983	Barming series + Malling series
Cox's Orange Pippin - 1984	Barming series
Golden Delicious - 1983	Langley series + Malling series
Golden Delicious - 1984	Langley series + Malling series

The soils of the orchards at the growers farms are described as "a fine sandy loam" (Discovery trees used in 1983, Chapter 2) and "Tunbridge Wells sands - Pembury series" (Cox's Orange Pippin trees used in 1983, Chapter 3).

Routine pest and disease spray programme at East Malling Research Station 1983 - 1985

Application Time	Chemical	Rate/hectare
Bud burst	dodine	2.8 l
Mouse ear	dodine	2.8 l
Green cluster	dithianon	2.1 l
	bupirimate	1.4 l
	phosalone	2.1 l
Pink bud	dithianon	1.4 l
	bupirimate	1.4 l
Flowering	thiophanate-methyl	2.2 l
Petal-fall	dithianon	2.1 l
	bupirimate	1.4 l
	phosalone	2.1 l
10 days after petal-fall	dithianon	1.4 l
	bupirimate	1.1 l
20 days after petal-fall	dithianon	1.4 l
	nitrothal-isopropyl	0.9 kg

Triadimefon (1 kg/ha) applied at 10 day intervals until shoot growth ceases.

end June (based on pheromone trap)	carbaryl	3.8 l
30 days after petal-fall	nitrothal-isopropyl	0.9 kg
early July (based on pheromone trap)	carbaryl	3.8 l

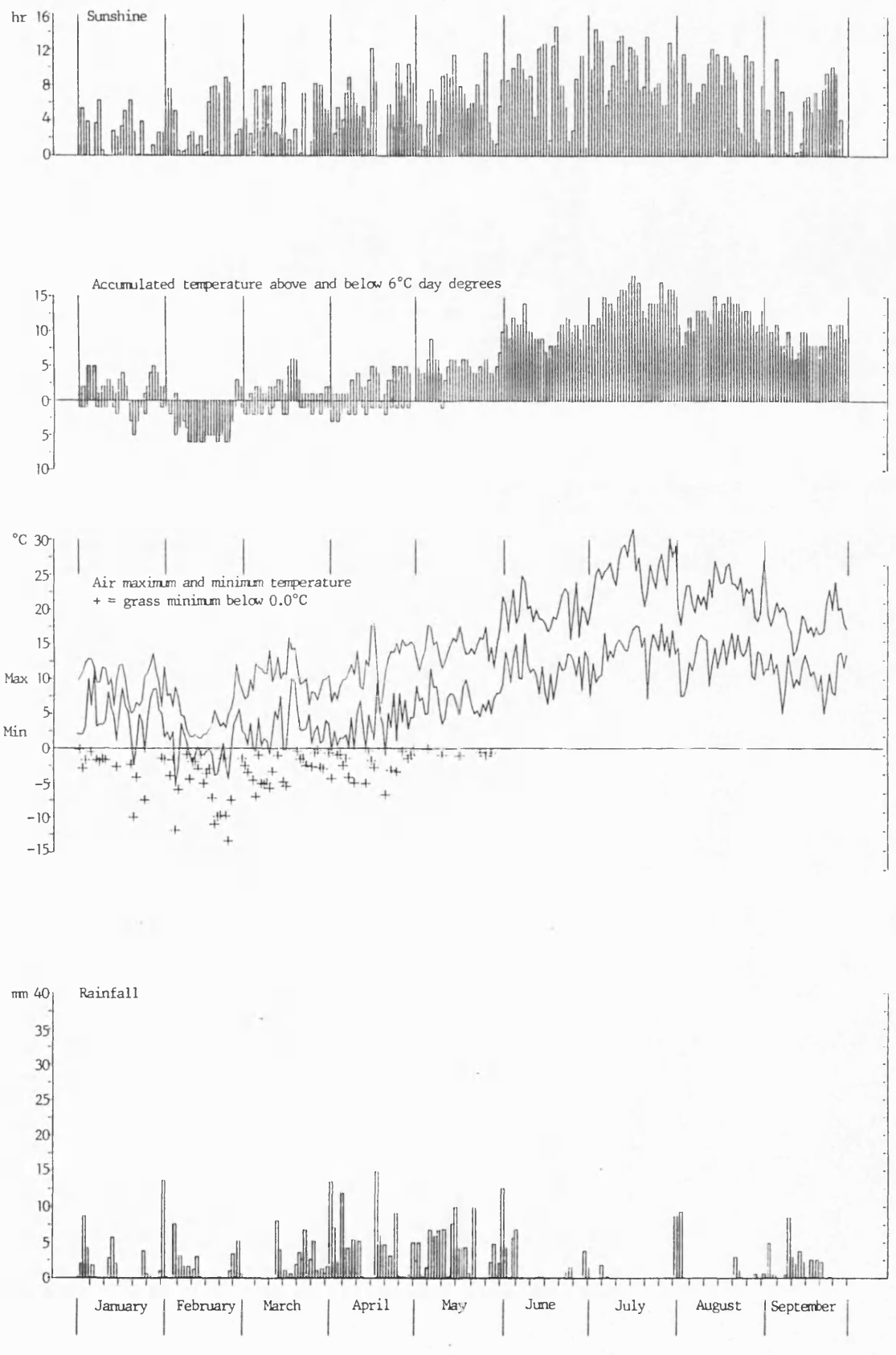
Routine pest and disease spray programme used on Discovery trees in 1983, Chapter 2

Application Time	Chemical	Rate/hectare
Mouse ear	dithianon	2.1 l
Green cluster	dithianon	2.1 l
	bupirimate	1.4 l
	pirimiphos-methyl	1.7 l
Pink bud	fenarimol	0.3 l
Petal-fall	fenarimol	0.5 l
7 days after petal-fall	fenarimol	0.6 l
	fenpropathrin	1.0 l
7 days later	bupirimate	1.1 l
7 days later	fenarimol	0.5 l
7 days later	fenarimol	0.5 l
	cypermethrin	0.3 l
10 days later	binapacryl	1.6 kg
10 days later	fenarimol	0.5 l
7 days later	binapacryl	1.4 kg
7 days later	triadimefon	0.1 kg

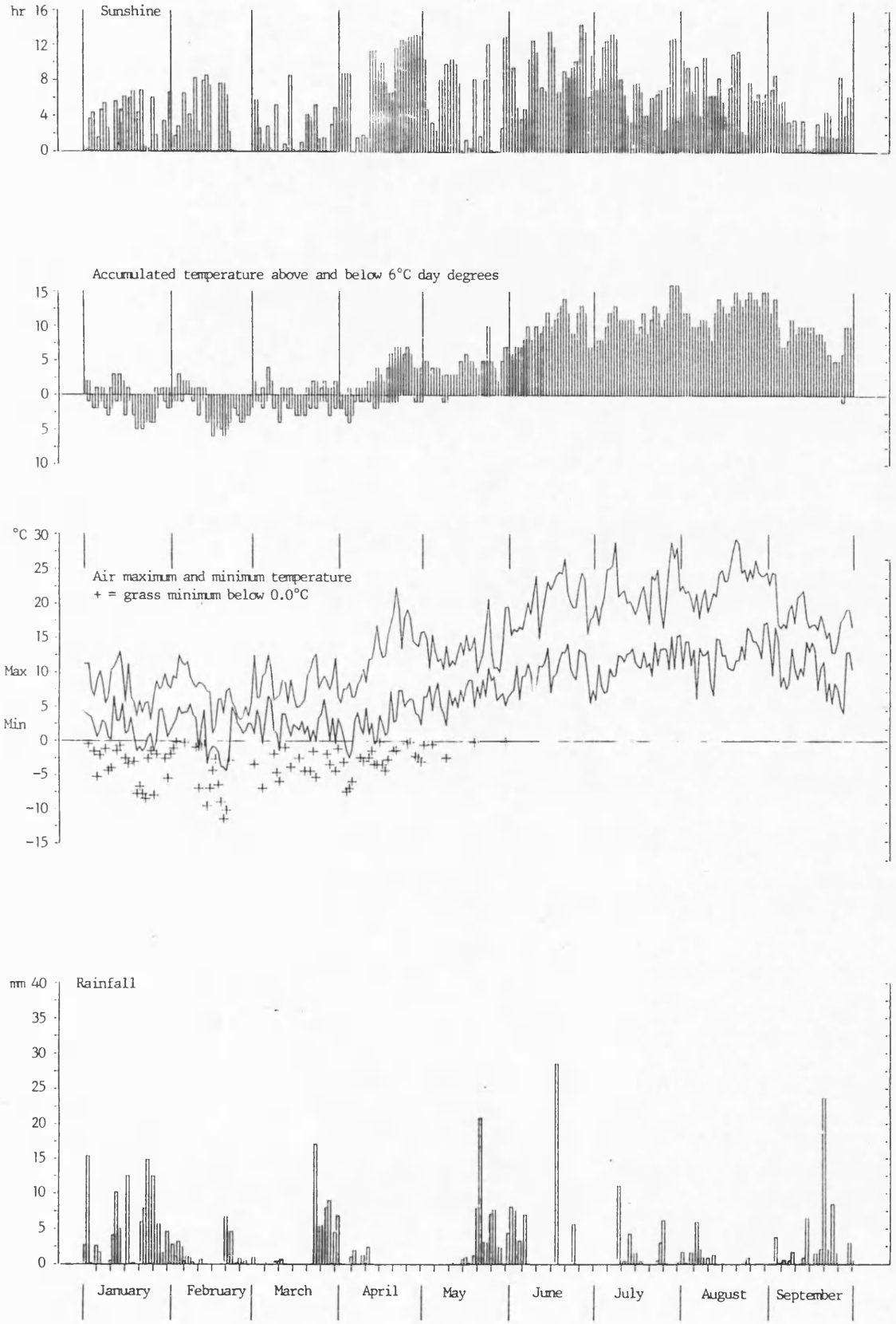
Routine pest and disease spray programme used on Cox's Orange Pippin trees at Horsmonden in 1983, Chapter 3

Application Time	Chemical	Rate/hectare
Bud burst	dithianon	2.1 l
Mouse ear	dithianon	2.1 l
Green cluster	pirimiphos-methyl	1.7 l
	triadimefon	0.1 kg
Pink bud	triadimefon	0.1 kg
Petal-fall and 6 further applications at 7 day intervals	fenarimol	0.5 l
7 days later	pirimicarb	0.4 kg
14 days later	cypermethrin	0.3 l
21 days later and 6 further applications at 7 day intervals	binapacryl	1.6 kg
7 days later	cyhexatin	0.7 l
7 days later	pirimicarb	0.4 kg

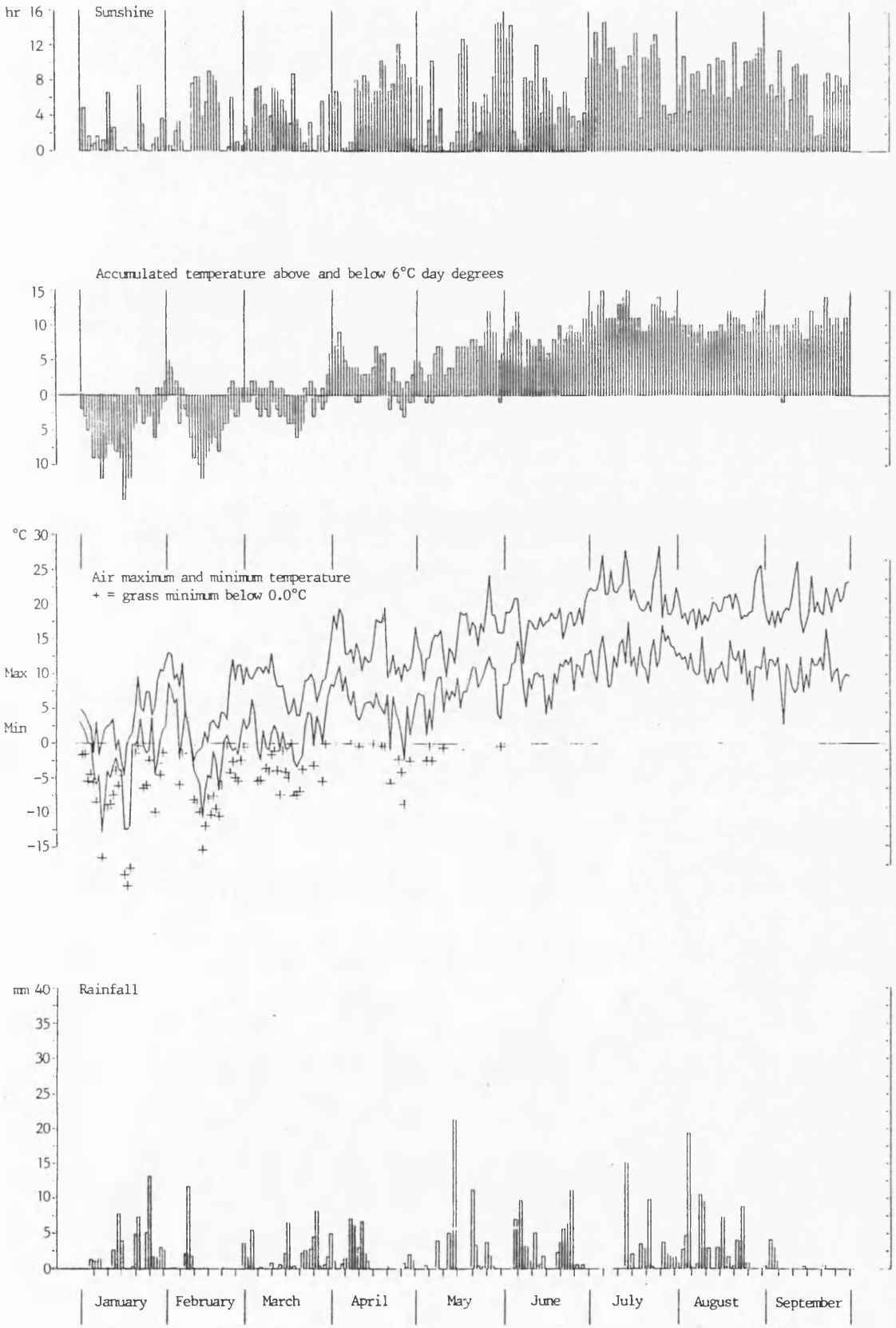
Meteorological records from East Malling Research Station - 1983


hr 16
12
8
4
0
Sunshine
Accumulated temperature above and below 6°C day degrees
15
10
5
0
5
10
°C 30
25
20
15
Max 10
5
Min
0
-5
-10
-15
Air maximum and minimum temperature
+ = grass minimum below 0.0°C
mm 40
35
30
25
20
15
10
5
0
Rainfall
January
February
March
April
May
June
July
August
September

Meteorological records from East Malling Research Station - 1984


hr 16
12
8
4
0
Sunshine
Accumulated temperature above and below 6°C day degrees
15
10
5
0
5
10
°C 30
25
20
15
Max 10
5
Min 0
-5
-10
-15
Air maximum and minimum temperature
+ = grass minimum below 0.0°C
mm 40
35
30
25
20
15
10
5
0
Rainfall
January
February
March
April
May
June
July
August
September

Meteorological records from East Malling Research Station - 1985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